MYCOPATHOLOGIA
ET
MYCOLOGIA APPLICATA

FUNDATORES:
R. CIFERRI
P. REDAELLI

EDENDA CURAVIT:
TIBOR BENEDEK
Chicago, Ill.

EDITORES:

F. de Almeida, São Paulo — A. N. Aravysky, Leningrad — A. E. de Arêa Leão, Rio de Janeiro — E. Baldacci, Milano — J. Bánhegyi, Budapest — E. Baudys, Brn — A. L. van Beverwijk, Baarn — K. B. Boedijn, The Hague — S. R. Bose, Calcutta — Wm. Bridge Cooke, Cincinnati, Ohio — H. Campins, Barquisimeto, Venezuela — A. L. Carrión, San Juan, P. R. — A. Castellani, Lisboa — A. Catanei, Alger — K. Cejp, Praha — A. Chaves Batista, Pernambuco — A. Ciccarone, Bari — N. F. Conant, Durham, N. C. — J. N. Couch, Chapel Hill, N. C. — J. Coudert, Lyon — S. N. Dasgupta, Lucknow — E. D. Delamater, Philadelphia — C. W. Dodge, St. Louis Mo. — C. W. Emmons, Washington, D.C. — E. Fejér, Budapest — G. W. Fischer, Pullman, Wash. — G. Florenzano, Firenze — A. da Fonseca, Rio de Janeiro — N. Fries, Uppsala — E. Gäumann, Zürich — A. Gonzalez Ochoa, Mexico, D.F. — H. Götz, München — L. Grigoraki, Marseille — E. I. Grin, Sarajevo — E. Hazen, Albany N.Y. — R. Heim, Paris — T. Hemmi, Kyoto — S. Ito, Sapporo — D. Janke, Marburg/Lahn — J. Jørstad, Oslo — P. N. Kashkin, Leningrad — F. D. Kern, State College, Pa — A. M. Kligman, Philadelphia, Pa. — C. da Silva Lacaz, São Paulo — J. Lodder, Delft — J. E. Mackinnon, Montevideo — O. de Magalhaes, Belo Horizonte — A. v. Mallinkrodt-Haupt, Köln — P. Martens, Louvain — D. S. Martin, Durham, N.C. — J. Meyer, Strasbourg — M. Pereiro Miguens, Santiago de Compostela — J. H. Miller, Athens, Ga. — R. Morquer Toulouse — E. M. Mrak, Berkeley, Calif. — J. A. Nannfeldt Upsala — P. Negroni, Buenos Aires — F. L. Niño, Buenos Aires — J. Obrtel, Praha — T. Ørskov, København — Pereira Filho, Porto Alegre — B. Peyronel, Torino — A. Pijper, Pretoria — A. Pilat, Praha — P. Pinetti, Cagliari — A. Pinto Viegas, Campinas — J. Politis, Athènes — G. Pollacci, Pavia — V. Puntoni, Roma — T. Rayss, Jerusalem — I. Reichert, Rehovot — F. Reiss, New York — E. Rivalier, Paris — D. P. Rogers, Urbana, Ill. — K. Sakaguchi, Tokyo — R. Sartory, Strasbourg — G. H. Scherr, Chicago — J. Schwarz, Cincinnati — F. J. Seaver, Winter Park, Fla. — G. Ségretain, Paris — R. Singer, Tucuman — A. H. Smith, Ann Arbor, Mich. — F. K. Sparrow, Ann Arbor, Mich. — J. A. Stevenson, Beltsville, Md. — Y. Takahashi, Sendai — R. V. Talice, Montevideo — M. J. Thirumalachar, Pimpri, Poona — A. Trotter Vittorio Veneto — N. van Uden, Lisboa — A. C. Ukil, Calcutta — R. Vanbreuseghem, Anvers — C. Vermeil, Tunis — O. Verona, Pisa — G. A. de Vries, Baarn — S. A. Waksman, New Brunswick, N. J. — J. Walker, London — L. E. Wehmeyer, Ann. Arbor, Mich. — J. Westerdijk, Baarn.

VOLUMEN XI

UITGEVERIJ Dr. W. JUNK — DEN HAAG — 1959

VOLUMEN XI

Fasc. 1-2 (pag. 1—176) edit: 15—IX—1959
Fasc. 3. (pag. 177—256) edit: 15—X—1959
Fasc. 4. (pag. 257—368) edit: 30—XI—1959
Supplementum edit: 15—XII—1959

INDEX AUTORUM

Alteras, I., 196.
Aravijsky, A. N., 143.
Baldacci, E., 250.
Benedek, T., 171, 172, 173, 253, 255, 368.
Benedek, Th. G., 253.
Boedijn, K. B., 354.
Bojalil, L. F., 287.
Bridge Cooke, Wm., 171, 252.
Burns Young, B., 297.
Caretta, G., 217.
Cerbon, J., 287.
Chaves Batista, A., 1.
Ciferri, R., 1.
Closs, G. L., 205.
Crosby, P. F., 109
Dominik, T., 359.
Durrell, L. W., 238.
Evolceanu, R., 196.
Fedors, P., 128.
Gabel, N. W., 205.
Hotchkiss, M., 297.
Kuehn, H. H., 109.
La Touche, Ch, J., 257, 277.
Lyon, I., 185.
Mains, E. B., 311.
Neuhauser, I., 254.
Pinetti, P., 155.
Rambelli, A., 135.
Rippon, J. W., 241.
Segretain, G., 327.
Singer, R., 170.
Stein, S. I., 205.
Trujillo, A., 287.
Verona, O., 103, 173.
Vicher, E. E., 185.
White, E. L., 185.
Widra, A., 177.
Zardetto di Toledo, O., 103.

Copyright 1959 by Uitgeverij Dr. W. JUNK, Den Haag

Printed in Holland by
Dijkstra's Drukkerij n.v., voorheen Boekdrukkerij Gebroeders Hoitsema, Groningen

CONTENTS: CONTENUTO: INHALT: TABLE DES MATIÈRES:

pag.

A. Chaves Batista & R. Ciferri: Sistemática dos fungos imperfeitos de picnostromas com himenio invertido (Peltasterales). (*Inst. de Micol., Univ. do Recife, Ist. Bot. e Lab. Crittogam., Univ. di Pavia*) 1

O. Verona & O. Zardetto de Toledo: Intorno ad un lievito osmofilo sistematicamente prossimo a *Sacch. elegans* Lodder et Kreger-van Rij. (*Ist. di.Microbiol. Agr. e Tech., Univ. di Pisa*) 103

Harold H. Kuehn & Paul F. Crosby: Growth and Nutrition of *Pseudoarachniotus roseus* in Submerged Cultures. (*Grain Processing Corp., Muscatine, Iowa*) 109

Peter Fedors: Inhibition of *Histoplasma capsulatum* and *Blastomyces dermatitidis* by *Pseudomonas aeruginosa* in vitro (*V.A. Hosp., Dawson Springs, Kentucky*) 128

Angelo Rambelli: Schede micologiche. III contributo. Micromiceti della Valle del Tevere. (*Centro di Sperim. Agric. e Forest., Roma*) 135

A. N. Aravijsky: Rare Mycological Findings in Pathological Material (*Dept. of Derm., First I. P. Pavlov Med. Inst., Leningrad*). 143

P. Pinetti: Rivista critica della letteratura micologica medica in Italia tra il 1946 ed il 1956. (*Clin. Derm., Centro di Micol., Univ. di Cagliari*). 155

O. Verona & Tibor Benedek: Iconographia Mycologica. (*Ist. di Patol. Veg. e di Microbiol. Agr. e Tecn., Univ. di Pisa, Mycol. Sect., Dept. of Derm., Stritch School of Med., Loyola Univ., Chicago, Ill.*) 173

Abe Widra: Studies on the Nucleus of *Candida* spp. (*Dept. of Bacteriol. and Immunol., Univ. of North Carolina, Chapel Hill, N.C.*). 177

E. E. Vicher, I. Lyon & E. L. White: Studies on the Respiration of *Trichophyton rubrum*. (*Dept. of Microbiol., Univ. of Illinois Coll. of Med., Chicago and Biol. Dept., Res. Labs., The Toni Co., Chicago, Ill.*). 185

R. Evolceanu & I. Alteras: Considérations à propos des caractères mycologiques et pathogéniques du *Keratinomyces ajelloi* Vanbreuseghem 1952, saprophyte de sol. (*Sect. Dermatol., Inst. de Thérapeut., Acad. de la Rép. Roumaine, Boucarest, Roumanie*) 196

Sam I. Stein, Gerhard L. Closs & Norman W. Gabel: Observations on Psychoneurophysiologically Significant Mushrooms. 205

G. Caretta: Caratteristiche morfo-biologiche di ceppi fungine del genere *Geotrichum* isolati da materiale umano. (*Centro di Micol. Med., Ist. Bot., Univ. di Pavia, Italia*) 217

L. W. Durrell & L. A. Schaal: The Nuclear Material in *Streptomyces*. . . 238

George H. Scherr & John W. Rippon: Experimental Histoplasmosis in Cold-blooded Animals. (*Dept. of Microbiol., Univ. of Illinois Coll. of Med., Chicago, Ill.*) 241

Obituary: Giovanni Battista Traverso 250

Announcement. 256

C. J. La Touche: Mouse Favus Due to *Trichophyton quinckeanum* (Zopf) MacLeod & Muende: A Reappraisal in the Light of Recent Investigations. (*Dept. of Dermatol., Gen. Infirm., Leeds and Dept. of Bacteriol., Univ. of Leeds, England*) 257

C. J. La Touche: Mouse Favus Due to *Trichophyton quinckeanum* (Zopf) MacLeod & Muende: A Reappraisal in the Light of Recent investigations. III. (*Dept. of Dermatol., Gen. Infirm., Leeds and Dept. of Bacteriol., Univ. of Leeds, England*) 277

L. F. Bojalil, A. Trujillo & J. Cerbon: Diferenciación bioquímica de algunas especies de Actinomycetes patógenos. (*Unidad de Patol., Esc. de Med., U.N.A.M. Hosp. Gen., Mexico* 7, *D.F., Mexico*). 287

Barbara Burns Young & Margaret Hotchkiss: Developmental Morphology in the Genus Nocardia. (*Dept. of Bacteriol., Univ. of Kentucky, Lexington, Ky., U.S.A.*) 297

E. B. Mains: Species of Hypocrella. (*Herbarium and Dept. of Bot., Univ. of Michigan, Ann Arbor, Mich., U. S. A.*). 311

G. Segretain: *Penicillium marneffei* n sp., agent d'une mycose du système réticulo-endothélial. (*Serv. de Mycol. et Physiol. Vég., Institut Pasteur, Paris, France*). 327

K. B. Boedijn: On Black an White Piedra. (s'-*Gravenhage*) 354

T. Dominik: Synopsis of a New Classification of the Ectotrophic Mycorrhizae Established on Morphological and Anatomical Characteristics. (*Forest Microbiol. Lab., Forest Res. Inst., Szczecin, Poland*) 359

Libri novi: 170, 252, 368

Gallery of Contemporary Noted Mycologists Nrs. 45 to 60.

Supplementum: O. Verona & T. Benedek: Iconographia Mycologica I: Plates A 1 - A 18, B 1 - B 10, C 1 - C 18, D 1 - D 4.

GALLERY OF CONTEMPORARY NOTED MYCOLOGISTS

45. O. Verona, Pisa.
46. G. A. de Vries, Baarn.
47. J. Schwarz, Cincinnati.
48. J. A. Nannfeldt, Uppsala.
49. S. Sakaguchi, Tokyo.
50. H. Götz, München.
51. Carlos da Silva Lacaz, Sao Paulo.
52. Alexander H. Smith, Ann Arbor.
53. E. Baldacci, Milano.
54. R. Heim, Paris.
55. A. Nannizzi, Siena.
56. E. Gäumann, Zürich.
57. Wm. Bridge Cooke, Cincinnati.
58. E. Baudijš, Brno.
59. A. González Ochoa, Mexico.
60. Y. Yasuda, Wakayama.

PLATES: TAVOLE: PLANCHES: TAFELN:

pag.

I. Giovanni Battista Traverso 250/251

Sample-plates of Iconographia Mycologica 176/177

Gallery Nrs. 45, 46, 47, 48, 49, 50, 51, 52 . . . . 176/177

Gallery Nrs. 53, 54, 55, 56 . . . . 256/257

Gallery Nrs. 57, 58, 59, 60 368

254 text-illustrations

SISTEMÁTICA DOS FUNGOS IMPERFEITOS DE PICNOSTROMAS COM HIMENIO INVERTIDO (PELTASTERALES)

A. Chaves Batista & R. Ciferri [1]

Publicação nº 72

(*Instituto de Micologia — Universidade do Recife*)

Êste trabalho reune as nossas observações, no periodo de alguns anos, sôbre os Fungos Imperfeitos, cujas frutificações se apresentam como picnostromas, de himenio invertido.

Essas observações, agora apoiadas na análise histologica de muitos especimens, de procedencias as mais diversas e sôbre os mais diferentes hospedeiros, permitiram-nos diferenciar dos fungos Sphaeropsidales aqueles que possuem himenio invertido, nos picnostromas, ou seja himenio desenvolvido na face interna da parede superior dessas frutificações assexuadas, em contraposição ao himenio basal.

A morfogenêse dêsses fungos é bem distinta no que toca á produção dos conidios, justificando assim considerá-los em uma outra ordem que denominamos como Peltasterales.

Por outro lado verificamos que a ocurrência dêsses fungos de picnostromas de himenio invertido está associada com os Ascomycetes da ordem dos Microthyriales, aparentemente funcionando como estádios metagenesicos das fórmas ascógenas.

Nenhuma prova nesse sentido, contudo, foi obtida até hoje, e isso obriga-nos a tratar êsses Fungos Imperfeitos como entidades autônomas, ainda quando intimamente relacionados com os ascostromas bem definidos dos Microthyriales, aceitando, nêsse particular, o consenso geral.

Guardados os limites de diversificação das familias de Microthyriales, em exata correspondencia, dividimos a ordem dos Peltasterales que abrange assim as seguintes novas familias: Peltasteraceae (Microthyriaceae), Asterinothyriaceae (Asterinaceae), Manginulaceae (Stigmateaceae), Plenotrichaceae (Micropeltaceae), Rhizothyriaceae (Polystomellaceae), Trichopeltulaceae (Trichopeltinaceae).

A ordem Peltasterales se degradaria entre os fungos de micélio esteril (Mycelia sterilia) representados pela familia Trichothallaceae, na qual o micélio é laminar, de hifas radiantes ou paralelas, sem picnostromas.

Do ponto de vista filogenetico as relações entre as familias de

---

[1] Diretor do Instituto Botânico e Laboratorio Criptogâmico da Universidade de Pavia, Itália.

Peltasterales obedeceriam uma linha evolutiva paralela ás das familias da ordem Microthyriales.

Neste trabalho, os gêneros cujas diagnoses deixamos de incluir mas que figuram nas chaves para as diversas familias aceitamo-los como valiosos, estritamente no sentido que lhes emprestaram os seus respectivos autores.

No desenvolvimento das pesquisas que deram origem a esta publicação contamos com a colaboração de numerosas Instituições Cientificas, especialmente quanto á cessão de especimens para estudo.

Os nossos agradecimentos sinceros são por isso oferecidos ao ,,Instituto Botânico della Universitá di Pavia'' Italia, ao ,,Természettudományi Muzeum Növénytir'' Hungria, ,,The University of Adelaide'' Australia, ,,Uppsala Universitets Institution för Systematisk Botanik'' Suecia, ,,National Herbarium of Victoria, South Yarra'' Australia, ,,The National Fungus Collections'' Maryland, Estados Unidos da America, ,,Bernice P. Bishop Museum'' Hawaii, ,,Djawatan Penjeldikan Alam'' Indonesia, ao ,,East African Agriculture and Forest Research Organisation'' Nairobi, ao ,,Department of Agriculture, Union of South Africa'', Pretoria e ao ,,Gana Mycological Herbarium'' Gana.

Igualmente, e com o melhor agrado, destacamos a ajuda recebida de nossos imediatos colaboradores, graças á qual logramos efetuar boa parte das investigações aqui focalisadas e preparar as cópias finais desta publicação.

Aos pesquisadores Dra. MARIA L. NASCIMENTO, Dr. A. FERNANDES VITAL e Dr. H. DA SILVA MAIA, e aos funcionários D. LIZETE DA S. PAIVA e MAURO WANDERLEY DE SIQUEIRA, todos do Instituto de Micologia, da Universidade do Recife, o nosso preito de reconhecimento.

Ao Dr. CARLOS ALBERTO AMARAL COSTA, da Faculdade de Medicina e Cirurgia do Pará, e estagiário da CAPES no Instituto de Micologia da Universidade do Recife, com quem estudamos e descrevemos alguns dos gêneros e especies de fungos Peltasterales, a nossa admiração pelo seu inteligente esfôrço.

PARTE GERAL

O estudo dos fungos imperfeitos dotados de picnidios escutelares é uma tarefa complexa, cheia de tropeços, pela heterogeneidade de criterios que em diferentes épocas varios micologos têm empregado.

De modo particular a documentação dispersa que se contém na literatura, ao lado de descrições parciais ou imprecisas de muitas especies de fungos desse grupo, que não ressaltam importantes caracteres de interesse da Sistemática, fazem com que o reconhecimento desses organismos nem sempre se processe com o devido rigor.

SACCARDO, em 1878 (Michelia II: 8; Syll. Fungorum, III: 625) criou a familia Leptostromaceae para compreender os fungos de picnidios pequeninos, dimidiados, escutelares, astomos, ostiolados ou de fenda longitudinal, membranaceos ou carbonaceos, negros, irrumpentes ou superficiais, tendo **Leptostroma** Fr. como tipo.

Tão larga amplitude necessariamente permitiria abrigar-se nessa famila uma série de fungos de caracteristicas biomorfologicas bem distintas.

VON HÖHNEL, em 1909 (Fragm. zur Myk. VIII: 79) considerando as relações dêsses fungos com os Ascomycetes dividiu a familia Leptostromaceae em duas secções, sem designá-las, entretanto.

Ainda VON HÖHNEL, em 1910 (Fragm. zur Myk. 518, pag. 59) a propósito de seu novo genero **Sirothyriella** criou a familia ,,Pycnothyrieen'' com picnidios escutelares radiados, de himenio invertido — picnotirios, destacando-a como associada aos Ascomycetes Microthyriaceae.

Em ,,Die Leptostromaceen'' DIEDICKE (Ann. Myc. XI: 72, 1913) aceitou a divisão de Leptostromaceae, como propuzera VON HÖHNEL.

SACCARDO, em 1913 em suas ,,Notae Mycologicae'' (Ann. Myc. XI: 315) criou o genero **Actinopelte,** com *A. japonica*, entre os Ascomycetes Microthyriaceae. Pouco depois, nesse mesmo ano de 1913, THEISSEN (,,Über einige Mikrothyriaceen'', Ann. Myc. XI: 507—508) demonstrou, ilustrando, que SACCARDO laborara num engano, pois o seu **Actinopelte** Sacc. é um fungo imperfeito de picnidios escutelares, radiados, com picnidiosporos, em vez de ascos e ascosporos; não indicou, porém, a sua posição sistemática.

NAUMOFF em 1915 em ,,Description des quelques nouvelles especes'' (Bull. Soc. Myc. France, 30: 428) propondo o novo gênero **Rhizothyrium** enquadrou-o entre os Pycnothyriaceae, ao lado dos generos **Sirothyriella** e **Actinopelte.**

Em 1923 VON HÖHNEL em seu ,,System der Fungi Imperfect Fuckel'' (*in* FALCKX, Mykol. Unters. & Ber. 1: 301—369) conservou Pycnothyriaceae distinta de Leptostromaceae, criando ainda a nova família ,,Peltopycnidieen VON HÖHN.'' para os picnídios radiantes que admitiu como fórmas imperfeitas de Trichopeltaceae, com o único gênero **Ischnostroma** SYD. Nesse mesmo trabalho (pag. 353) propoz ainda a família ,,Actinothyrieen v. H.'' entre os Tuberculariaceae, porém sem diferençá-la, propriamente, dos Pycnothyriaceae.

In ,,Ueber die Gattungen **Actinopelte** SACC.'' PETRAK (Ann. Myc. XXII: 53—55, 1924) sem aludir ao ,,System'' de VON HÖHNEL elegeu a família Actinopeltaceae, monotípica, baseada em **Actinopelte.** Mais tarde, em 1925, VON HÖHNEL, em trabalho *post mortem*, publicado por WEESE (Mitt. Bot. Inst. Techn. Hochsch., Wien, 2: 32) reconheceu que a sua família Actinothyriaceae talvez fosse Pycnothyriaceae, mas a manteve, entretanto, ao lado de Tuberculariaceae, com os gêneros de **Actinothyrium** G. KUNZE, **Actinopelte** SACC. e **Columnothyrium** BUBÁK.

Não obstante todas essas opiniões, CLEMENTS & SHEAR (1931, em ,,The Genera of Fungi") consideraram somente a família Leptostromaceae, desprezando as suas divisões que já se impunham, com uma revisão. A obra dêsses Autores que ainda recentemente foi reeditada (1954), tornar-se-ia clássica nos trabalhos de sistemática micológica, contribuindo, por outro lado, para o incremento do número de pesquisadores da taxonomia, sem ajudar, entretanto, ao progresso do conhecimento dos fungos Leptostromaceae, que trataram então reunidos a outros fungos de características bem definidas.

Em 1940, TEHON poz em evidência uma vez mais, a importância dos picnídios de himênio invertido — picnotírios — para a sistemática dos fungos imperfeitos (Trans. Illinois State Acad. Sci. 33: 63—65), propondo então uma nova ordem Pycnothyriales, com as famílias Pycnothyriaceae e Rhizothyriaceae, esta sendo um desmembramento de Pycnothyriaceae VON HÖHNEL, para abranger aos fungos de picnídios superficiais, desprovidos de micélio livre, escutelares, radiados, de himênio invertido, com columela em conexão com o micélio interno ou hipostroma, designado ,,rizotírio"; a essa família Rhizothyriaceae foram agregados os gêneros **Rhizothyrium** NAUM. e **Actinothyrium** G. KUNZE e sugerido o seu parentesco com os Polystomellaceae, pela presença de hipostroma.

LIMBER & CASH, em 1945, estudando *Actinopelte dryina* (Mycologia, 37: 129—137) ressaltam a família Rhizothyriaceae TEHON, concordando que Actinothyriaceae VON HÖHNEL como fôra criada ficou indistinguível de Pycnothyriaceae.

BESSEY em seu livro ,,Morphology and Taxonomy of Fungi" 1952, indica, à pag. 581, a proposição de TEHON há pouco lembrada, admitindo assim a conveniência de desmembramento da família Leptostromaceae.

AINSWORTH & BISBY em ,,A Dictionary of Fungi" (ed. 1954) não registam as famílias Pycnothyriaceae e Rhizothyriaceae, igualmente assim procedendo MARTIN, em sua ,,Key to the families of Fungi" como apêndice, anexada na parte final dêsse Dicionário.

Incontestavelmente, a família Leptostromaceae não pode continuar come se encontra; AINSWORTH & BISBY relacionam 89 gêneros, dos quais 49 como monotípicos, como a ela pertencentes, inexistindo, porém, razóaveis elementos de coordenação para a Sistemática, pois nessa família situam-se fungos de morfologia a mais variável além de, filogeneticamente, muito afastados uns dos outros.

Sugerimos então, apoiados nas observações que nos vêem desde VON HÖHNEL (1909) destacar dos Leptostromaceae os fungos de picnídios escutelares de himênio invertido e os de micélio laminar radiado com picnotírios ou picnídios formados por picnose.

Tais fungos serão reunidos numa nova ordem — PELTASTERALES — que compreenderá as famílias Peltasteraceae, Asterinothyriaceae, Manginulaceae, Plenotrichaceae, Rhizothyriaceae e Trichopeltulaceae, cujos caracteres focalizaremos adiante.

O fracionamento em tela da família Leptostromaceae segue as linhas gerais das idéias de TEHON, que não se apoiaram, entretanto, nas Regras Internacionais de Nomenclatura, uma vez que a ordem Pycnothyriales, dêsse autor, tinha como tipo a família Pycnothyriaceae, que fez derivar, por seu turno, do gênero **Pycnothyrium.**

Acontece exatamente porém, que **Pycnothyrium** DIED. foi reconhecido, em 1918, como sinônimo de **Diplopeltis** PASS. por VON HÖHNEL (Frag. zur Mykol. CCLXXX, em Ann. Mycol. XVI: 171) e, depois, **Diplopeltis** identificado como sinônimo, igualmente, do antigo gênero **Pycnoseynesia** O. KUNTZE (ver AINSWORTH & BISBY, l.c.) que passou a prevalecer assim, sôbre os demais, consoante a lei de prioridade.

O gênero **Pycnothyrium** foi criado no mesmo trabalho em que DIEDICKE publicou os resultados de sua revisão do gênero **Diplopeltis** PASS., ao examinar o especimen tipo dêsse gênero que lhe fôra cedido por SACCARDO, quando verificou então que os picnidiosporos, em vez de bicelulares e marrons, como os descrevera PASSERINI, são unicelulares e hialinos, formados em picnídios com himênio invertido. Não obstante VON HÖHNEL tê-lo identificado com o gênero **Diplopeltis** (1918, l.c.) o que reafirmou depois em 1923 (System der Fungi, etc.) continuou indevidamente o uso de **Pycnothyrium.**

Excluído **Pycnothyrium,** por sua condição de sinonímia, não poderia êsse gênero, dessa fórma, servir de tipo a uma família e consequentemente, a uma nova ordem.

Aliás, o nome Pycnothyriaceae fôra inicialmente empregado por VON HÖHNEL (1910, l.c.) conforme já expuzemos, mas, nesse caso, como se deu também com a sua família Peltopycnidiaceae a família não descendeu de um gênero tipo, pois ainda DIEDICKE não criara o seu **Pycnothyrium**; o nome resultara assim, simplesmente, da designação da frutificação imperfeita, invertida — picnotírio.

De acôrdo com a experiência que adquirimos no trato dos fungos Microthyriales e de suas fórmas imperfeitas, de par com o nosso estudo sôbre a organogênese dos Trichopeltinaceae, elegemos a nova ordem PELTASTERALES entre os Fungi Imperfecti, porém filogeneticamente associada àquela ordem dos Ascomycetes, para conter as frutificações picnostromaticas, escutelares, uni ou pluriloculares, de himenio invertido, destituidas de micélio, providas de micélio livre, ou laminar, que representam os Micropeltaceae, Polystomellaceae, Asterinaceae, Microthyriaceae, Stigmateaceae, Trichopeltinaceae em seu estágio assexuado, conforme admitido em geral.

PARTE SISTEMÁTICA

Ord. PELTASTERALES — Typus Fam. Peltasteraceae nobis.

Fungi commensales vel plantarum parasitici. Mycelium superficiale nullum, vel ex hyphis liberis vel laminatis compositum. Pycnostromata superficialia vel subcuticularia, vel cum hypostromatis; hymenium inversum; scutata libera, vel ob pellicula mycelica tecta, pycnosice efformata. Pycnidiosporae variae.

Compreende essa nova ordem as seguintes familias: Peltasteraceae, Asterinothyriaceae, Manginulaceae, Plenotrichaceae, Rhizothyriaceae, Trichopeltulaceae.

No quadro anexo indicamos as relações de afinidade entre as familias da ordem Peltasterales e as familias da ordem Microthyriales que sugerem, até certo ponto, uma constituição divergente ou paralela dos Peltasterales, a terminar nas formas estéreis de Trichothallaceae.

ORDEM MICROTHYRIALES	MICROTHYRIACEAE	Peltasteraceae
	ASTERINACEAE	Asterinothyriaceae
	STIGMATEACEAE	Manginulaceae
	MICROPELTACEAE	Plenotrichaceae
	POLYSTOMELLACEAE	Rhizothyriaceae
	TRICHOPELTINACEAE	Trichopeltulaceae

ORDEM PELTASTERALES

Mycelia sterilia

TRICHOTHALLACEAE

CHAVE PARA AS FAMILIAS

A) Destituido de micelio.
B) Picnostromas superficiais — Rhizothyriaceae
BB) Picnostromas subcuticulares — Manginulaceae.
AA) Destituido de micelio, ou, se presente, sem hifopodios — Peltasteraceae.
AAA) Micelio presente, com hifopodios.
C) Com picnostromas.
D) Micelio livre lamelar — Trichopeltulaceae.
DD) Micelio livre peliculoso — Plenotrichaceae.
CC) Sem picnostromas — Trichothallaceae.

Peltasteraceae n. fam.

Micélio livre ausente ou constituido por hifas marron, não hifopodiadas; picnostromas marron-negros, escutelares, radiados, ostiolados ou não, sem hipostroma; himenio invertido; esporos de várias formas, hialinos ou corados. Tipo *Peltaster* SYD.

Mycelium liberum nullum vel ex hyphis brunneis, non hyphopodiatis compositum. Pycnostromata scutata, atro-radiata, ostiolata vel non; hymenium inversum; hypostromata nulla efformata. Sporae variae. Typus *Peltaster* SYD.

Filiam-se a essa familia algumas fórmas imperfeitas frequentemente atribuidas aos Microthyriaceae.

CHAVE PARA OS GÊNEROS

HIALOAMEROSPOROS

A) Micelio livre ausente.

B) Picnostromas radiados de disposição não circinada.

C) Pseudoostiolados; picnidiosporos catenulados .... **Catenulaster** BAT. & COSTA

CC) Dehiscencia estelar; hymenio exibindo curtos conidioforos; picnostromas rotundos, simples.

D) Picnidiosporos pleurogenos .... **Acarella** SYD.
(Ann. Myc. XXV: 123, 1927)

DD) Picnidiosporos acrogenos .. **Pycnoseynesia** O. KUNTZE
(Rev. Gen. Plant. I: 508, 1891)

BB) De disposição circinada; picnidiosporos acrogenos .... **Diedickea** SYD.
(Ann. Myc. XI: 266, 1913)

AA) Micelio livre presente, reticulado; picnostromas radiados; picnidiosporos sesseis.

E) Dehiscencia estelar .... **Peltaster** SYD.
(Ann. Myc. XV: 261, 1917)

EE) Ostiolados .... **Elachopeltis** SYD.
(Ann. Myc. XXV: 121, 1927)

AAA) Micelio livre submembranoso, fumagineo; picnostromas radiados; ostiolados; picnidiosporos sesseis .... **Eriothyrium** SPEG.
(Fung. Fueg. N⁰ 426, 1887)

FEOAMEROSPOROS

A) Micelio livre presente, reticulado; picnostromas radiados.

B) Ostiolados; picnidiosporos sesseis .**Asteroscutula** PETRAK
(Sydowia, II: 59, 1948)

BB) Dehiscencia estelar; picnidiosporos sesseis .... **Asterostomula** THEISS.
(Ann. Myc. XIV: 270, 1916)

HIALODIDIMOSPOROS

A) Micelio livre reticulado; picnostromas radiados; dehiscencia estelar.
B) Conidioforos nodulares, paliçadiços
.... **Thyriostromella** BAT. & COSTA
BB) Conidioforos ausentes .... **Mindoa** PETRAK
(Sydowia III: 248, 1949)

FEODIDIMOSPOROS

Micelio livre, reticulado; picnostromas radiados; dehiscencia estelar .... **Leprieurina** ARN.
(Ann. Agr. Montp. XVI: 210, 1918)

HIALOFRAGMOSPOROS

Micelio livre ausente; picnostromas radiados; ostiolados
.... **Septothyrella** v. HÖHN.
(Sitzb. Akad. Wien. CXX, 393, 1911)

FEOFRAGMOSPOROS

A) Micelio livre, reticulado; picnostromas radiados.
B) Dehiscencia glebosa; conidioforos curtos
.... **Allothyriella** BAT.; CIFERRI & NASC.
BB) Ostiolados; picnidiosporos sesseis
.... **Allothyriopsis** BAT.; CIFERRI & MAIA

FEODICTIOSPOROS

Micélio livre, escasso; picnostromas radiados; ostiolados; picnidiosporos sesseis .... **Lasiothyrium** SYD.
(Philipp. J. Sci. VIII: 503, 1913)

FEOSCOLECOSPOROS

Micélio ausente; picnostromas prosenquimáticos, radiados; dehiscencia glebosa; picnidiosporos multiseptados
.... **Stigmopeltella** SYD.
(Ann. Myc. XXV: 130, 1927)

Catenulaster BAT. & COSTA, n. gen.
Tipo — *C. anacardicola* nobis.

Desprovido de micélio livre. Picnostromas superficiais, escutelares, dimidiados, membranosos, olivaceo-marron, glabros, radiados. Himenio invertido. Picnidiosporos bacilares ou cilindraceos, continuos, catenulados, hialinos.

Mycelio libero nullo. Pycnostromata superficialia, scutata, dimidiata, membranosa, olivaceo-brunnea, glabrata, radiata. Hymenium inversum. Pycnidiosporae bacillares vel cylindraceae, continuae, catenulatae, hyalinae.

Catenulaster anacardicola Bat. & Costa n. sp.

Ausencia de micélio livre. Picnostromas superficiais, epifilos, isolados ou gregários, facilmente destacaveis da matriz, orbiculares, membranáceos, escutelar-dimidiados, marron, com 90—110 μ de diametro e 11.5—26.5 μ de altura, glabros, pseudo-ostiolados; margens sub-hialinas, reticuladas, peliculosas, até 27 μ de extensão; parede superior radiada, prosenquimática, com 3.5—6 μ de diametro, formada por celulas retangulares, de 3.5—5 × 2.5—3.4 μ; parede inferior indistinta. Himenio invertido. Ausencia de hipostroma. Conidioforos ausentes. Picnidiosporos bacilares, continuos, retos, catenulados, sesseis, com 3.5—4.5 × 1.2 μ hialinos, reunidos por uma substancia mucoide.

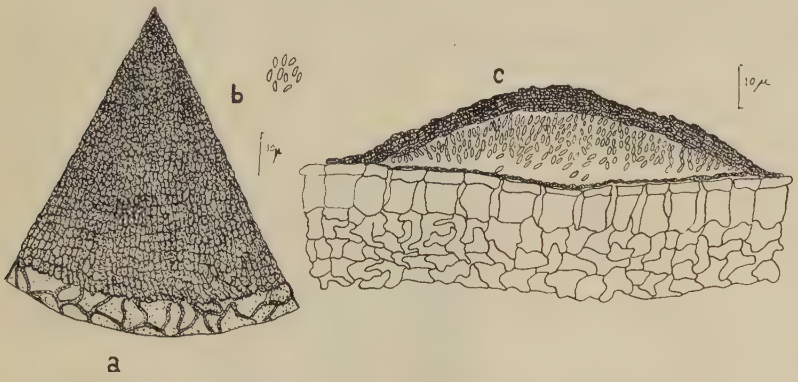


Fig. 1. *Catenulaster anacardicola* Bat. & Costa n. sp.
a) sector da parede superior de picnostroma; b) picnidiosporos; c) picnostroma sob córte longitudinal.

Sôbre folhas vivas de *Anacardium occidentale* L. associado a *Plenotrichella anacardii* Bat. & Costa. Jaboatão. Leg. Prof. Hermano Castro, 18.1.1957. Tipo, 5983, Instituto de Micología, Universidade do Recife.

Mycelio libero nullo. Pycnostromata superficialia, epiphylla, sparsa vel gregaria, facile secedentia, orbicularia, scutatodimidiata, 90—110 μ diam., 11.5—26.5 μ alt., brunnea, glabrata, pseudo-ostiolata; marginibus pelliculosis, subhyalinis, usque 27 μ longis; pariete superne radiato, prosenchymatico, 3.5—6 μ cr., ex cellulis rectangularibus, 3.5—5 × 2.5—3 μ efformato; pariete basali inconspicuo. Conidiophoris non visis. Pycnidiosporae bacillares, continuae, rectae, catenulatae, sessiles, 3.5—4.5 × 1.2 μ, hyalinae, in mucocongestae.

In foliis vivis *Anacardii occidentalis* L. socia *Plenotrichellae anacardii* Bat. & Costa Jaboatão — Leg. Prof. Hermano Castro 18.1.1957. Typus, 5983, Instituto de Micologia, Universidade do Recife — Pernambuco — Brasil.

Acarella Syd.

in Sun. Mycol. XXV: 123, 1927.

Micélio livre ausente. Picnostromas superficiais, dimidiado-escutelares, radiados, oliva-marron, de dehiscência irregular.

Pycnoseynesia myriadea (Syd.) n. comb.

Sin.: *Pycnothyrium myriadeum* Syd. *in* Ann. Mycol. XIV: 371, 1916.

Micélio livre ausente. Picnostromas lamelares, superficiais, planos, orbiculares ou irregulares, 1—2 mm de diam., gregários ou confluentes, membranoso-coriáceos, marron-negros, de contexto meandriforme-radiado, de margens nitidamente franjadas, onde se destacam então hifas cilíndricas, septadas, com células de 8.5—11 × 1.5—3.5 µ, às vezes intercruzadas, de modo a constituir delicada película. Lóculo picnidial ocupando quase toda a extensão do picnostroma, 500—1200 µ de comprimento e 44—58 µ de altura.

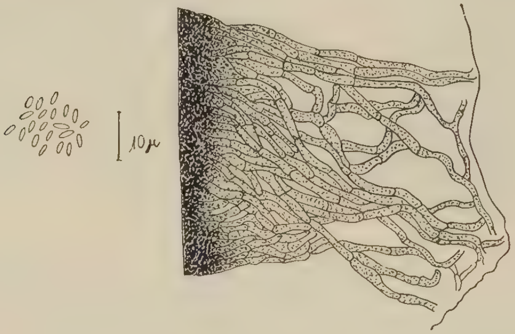


Fig. 2. *Pycnoseynesia myriadeum* (Syd.) Batista & Ciferri n. comb. Porção franjada, marginal ao picnostroma e picnidiosporos.

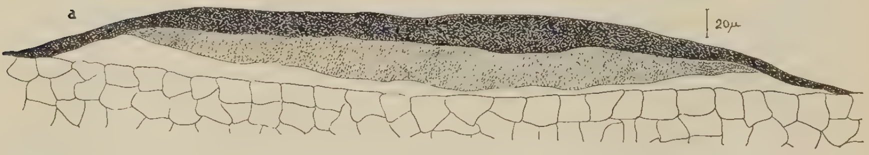


Fig. 3. *Pycnoseynesia myriadeum* (Syd.) Bat. & Ciferri n. comb. Picnostroma em secçao longitudinal.

Himênio invertido; parede basal gelatinosa, indistinta. Conidióforos muito curtos, quasi imperceptíveis. Picnidiosporos numerosíssimos, enchendo toda a cavidade do picnostroma, cilindráceos, retos, contínuos, hialinos, 1-gutulados ou não, 3—4.5 × 1—1.5 µ.

Sôbre colmos mortos de *Bambusa vulgaris*. Los Bãnos, Laguna, Luzón, Filipinas, Out. 1913, Fungi Malayani, C. F. Baker, nº 581.

Det. A. CHAVES BATISTA e M. LOURDES NASCIMENTO.

Obs.: O especimen em causa, aparentemente parte do tipo de SYDOW, foi-nos cedido, generosamente, pelo Dr. BOHUS GABOR, do Magyar Nemzetti Museum, Hungria, a quem agradecemos, assim, a oportunidade de reexaminar e redescrever esta espécie de **Pycnoseynesia.**

Allothyriella BAT. & CIF. & NASC. n. gen.

Tipo: *A. marcgraviae* n. sp.

Micélio livre reticulado-ramificado, marron-negro, sem setas, e sem hifopodios. Picnostromas superficiais, escutelares, orbiculares, marron-negros, radiados, de margens franjadas e dehiscencia glebosa, uniloculares; parede basal inconspicua. Conidioforos prismatico-truncados, hialinos, continuos, em himenio invertido. Picnidiosporos acrogenos, cilindraceos, pluri-transverso-septados, marron.

Mycelio libero reticulato-ramoso, atro-brunneo, non setoso, sine hyphopodiis. Pycnostromata superficialia, scutata, orbicularia, atro-brunnea, radiata, marginibus pelliculosis, glebose dehiscentia, unilocularia; pariete basali inconspicuo. Hymenium inversum. Conidiophoris prismatico-truncatis, hyalinis, continuis. Pycnidiosporae acrogenae, cylindraceae, pluritransversaliter septatae, brunneae.

Obs.: Êste genero é, provavelmente, a fórma imperfeita de **Allothyrium** SYD.

Allothyriella marcgraviae BAT. & CIFERRI & NASC. n. sp.

Micélio livre superficial, hipófilo, em colonias circulares, pequeninas, 4—10 mm de diam, isoladas ou confluentes; hifas reticulado-ramificadas, sinuosas, septadas, não constrictas, com células de 7.5—20 × 2.5—4.5 μ sem setas e sem hifopodios. Picnostromas superficiais, gregários, ás vezes confluentes, hipofilos, escutelares, orbiculares, 165—220 μ diam, 55—61 μ de alt., marron-negros, uniloculares; parede superior radiada, formada por células retangulares de 5,5—11 × 3—7 μ, de dehiscência glebosa e margens peliculosa-franjadas, até 50 μ de extensão, onde se destacam hifas de 3,5—5 μ de diam. Himenio invertido; parede basal inobservada. Conidioforos prismatico-truncados, continuos, 9.5—11 × 3.5—4.5 μ, hialinos. Picnidiosporos acrogenos, cilindraceos, de polos rotundos, a principio hialinos, continuos, depois 3-transverso-septados, não constrictos, marron, 31—41 × 6—7.5 μ.

Sôbre folhas de *Marcgravia rectiflora* FR. & PLANCH. Fazenda San Antonio, pr. Barros, Tungurahua, Equador. Leg. H. SYDOW, 7, Jan. 1938. Tipo, 1211, SYDOW, Fungi exotici exsiccati, no Magyar Nemzetti Museum, Hungria.

Obs.: A espécie em foco, que é o tipo do novo gênero **Allothyriella,** aparentemente será a fórma imperfeita de *Allothyrium*

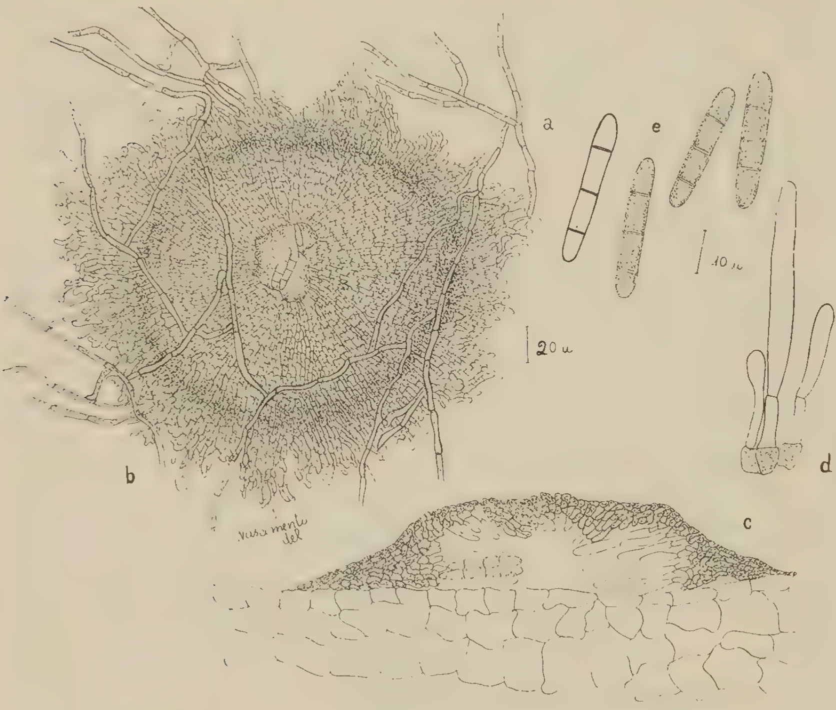


Fig. 4. *Allothyriella marcgraviae* Bat. & Nasc. n. sp.
a) Micélio livre; b) picnostroma sob vista frontal; c) córte longitudinal de picnostroma revelando himenio invertido; d) conidióforos, e picnidiosporos imaturos; e) picnidiosporos jovens e maduros.

marcgraviae Syd. O seu assinalamento é consequência do estudo do especimen de Sydow que obtivemos para análise contando com a colaboração do Dr. Bohus Gábor, da Hungria. Coincidentemente, não logramos dessa exsicata uma unica frutificação de **Allothyrium** enquanto passou desapercebida a Sydow a presença dêsse provavel estágio imperfeito, de picnostromas.

Mycelio libero superficiale, hypophyllo, plagulis circularibus, minutis, 4—10 mm diam., vel confluentibus, hyphis reticulato-ramosis, sinuosis, septatis, non constrictis, ex cellulis 7.5—20 × 2.5—4.5 μ non setosis, non hyphopodiatis, compositis. Pycnostroma superficialia, hypophylla, gregaria vel confluentia, scutata, orbiculata, 165—220 μ diam., 55—61 μ alt., atro-brunnea, unilocularia; pariete superne radiato, ex cellulis rectangularibus, 5.5—11 × 3—7 μ, glebose dehiscentia, marginibus pelliculoso-fimbriatis usque 50 μ long., ex hyphis 1.5—5 μ cr. efformato. Hymenium inversum; pariete basali non viso. Conidiophoris prismatico-truncatis, continuis, hyalinis, 9.5—11 × 3.5—4.5 μ. Pycnidiosporae acrogenae, cylindraceae, ad polos rotundatae, primo continuae, hyalinae, dein

3 transversaliter septatae, non constrictae, brunneae, 31—41 × 6—7.5 μ.

In foliis *Marcgraviae rectiflorae* Tr. & Planch. Hacienda San Antonio, pr. Baños, Tungurahua, Ecuador, Leg. H. Sydow, 7 Jan. 1938. Typus, 1211, Magyar Nemzetti Museum, Hungria.

Allothyriella tecleae Bat. & Nasc., n. sp.

Micélio livre, superficial, hipófilo, de hifas marron, onduladas, septadas, tendo células de 5.5—27 × 2.7—5 μ, sem setas e sem hifopódios; êsse micélio é comum a *Echidnodes tecleae* (Doidge) Bat. & Nas. Picnostromas superficiais, hipófilos, gregários, numerosos, escutelar-orbiculares, 230—270 μ de diam., ástomos, de dehiscência glebosa-estelar, membranosos, sub-parenquimáticos, de parede superior radiada, formada por células retangulares de 4.5—6 × 1.5—4 μ; margens franjadas, com hifas de 5.5—10 μ de extensão. Himênio invertido; parede basal inobservada. Conidióforos papiliformes ou prismáticos, contínuos, hialinos, 5—10 × 5 μ. Picnidiosporos acrógenos, cilindráceo-alongados, a princípio hialinos, depois marron, 3—7-transverso-septados, não constrictos, 45—51 × 3.5—5 μ.

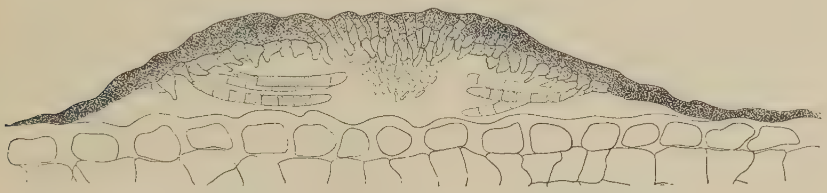


Fig. 5. *Allothyriella tecleae* Bat. & Nasc.
Picnostroma sob córte transversal e picnidiosporos.

Sôbre folhas de *Teclea nobilis* associado a *Echidnodes tecleae* (Doidge) nobis. Entebbe Road, Uganda. Leg. C. G. Hansford, Nov. 1943. Tipo, Mycol. Herb. 34783, Dep. Agr. Union of South Africa.

Mycelium liberum superficiale, hypophyllum, ex hyphis brunneis, undulatis, septatis, ex cellulis 5.5—27 × 2.7—5 μ, non setosis, non hyphopodiatis, compositum. Pycnostromata superficialia, hypophylla, numerosa, gregaria, scutato-orbiculata, 230—270 μ diam., gleboso-stellata dehiscentia; pariete superiore membranoso, subparenchymatico, ex cellulis rectangularibus, 4.5—6 × 1.5—4 μ, radiis dispositis; marginibus fimbriatis, ex hyphis usque 5.5—10 μ longis; hymenium inversum; pariete basali non viso. Conidiophoris papilliformibus vel prismaticis, continuis, hyalinis, 5—10 × 5 μ. Pycnidiosporae acrogenae, cylindraceo-elongatae, primo hyalinae dein brunneae, 3—7 transversaliter septatae, non constrictae, 45—51 × 3.5—5 μ. — In foliis herbarii *Tecleae nobilis*, socia *Echidnodis tecleae* (Doidge) Bat. & Nasc. Entebbe Road, Uganda.

Leg. C. G. HANSFORD, Nov. 1943. Typus, 34783, Mycol. Herb. Dep. Agric. Union of South Africa.

Obs.: DOIDGE (loc. cit.) descreveu os picnidiosporos como hialinos; encontramo-los predominantemente hialinos, por imaturidade, e marron, quando maduros.

Allothyriopsis BAT. & CIFERRI & MAIA n. gen.
Tipo: *A. landolphiae* n. sp.

Micélio livre reticulado, marron, não setoso, sem hifopodios. Picnostromas escutelares, marron, radiados, ostiolados, uniloculares, Himenio invertido. Picnidiosporos sesseis, 3- ou mais septos transversais, olivaceo-marron.

Mycelio libero reticulato, ex hyphis brunneis, non setosis, non hyphopodiatis, composito. Pycnostromata scutata, brunnea, radiata ostiolata, uniloculata. Hymenium inversum. Pycnidiosporae sessiles, 3-pro more transversaliter septatae, olivaceo-brunneae.

Allothyriopsis landolphiae BAT. & CIFERRI & MAIA n. sp.

Plágulas hipofilas, circulares a sub-circulares, isoladas ou confluentes — 3—7 mm de diam. Micélio superficial não setoso de hifas telemorficas, septadas, não hifopodiadas, de ramificações em angulos de 45°, alterna ou unilateralmente, com células de 6—12.5 × 2—2.5 μ larg. Haustórios nao vistos. Picnostromas escutelares, orbiculares, marrons, 120—145.5 × 21.5—32.5 μ alt., ostiolados, 10—12.5 μ diam. paredes 10—13.5 μ larg.; parede superior com células retangulares, radialmente dispostas, 4—5 × 3—4 μ e bordos franjados com células hifais de 12.5—19 × 1—2 μ de diam. Himenio simples, invertido; parede basal inconspicua. Conidioforos ausentes. Picnidiosporos cilindraceo-fusoides 3-septados, constrictos de polos agudos, a principio hialinos, depois olivaceos, 19—20 × 2.5—3 μ.

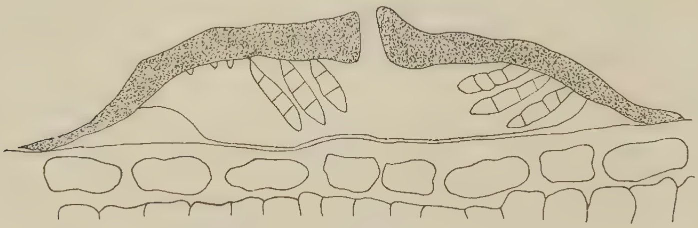


Fig. 6. *Allothyriopsis Landophiae* BAT. & MAIA n. sp.
Picnostroma sob córte longitudinal.

Sôbre folhas de *Landolphia owariensis* associado a *Asterinella sp.* (imaturo) Aburi, Ghana, Leg. S. J. HUGHES, 24.5.949. Tipo, nº 875, em Ghana Mycol. Herb.

Plagulae hypophyllae, rotundatae, isolatae, vel non, 3—7 mm diam. Mycelium superficiale, reticulato, ex hyphis alternatim vel unilateraliter ramosis in angulis 45°, septatis, ex cellulis 6—12.5 ×

2—2.5 μ, non setosis, non hyphopodiatis, compositum. Pycnostromata scutata, orbiculata, brunnea, 120—145.5 μ diam, 21.5—32.5 μ alt., ostiolata, 10—12.5 μ diam.; pariete superiore 10—13.5 μ cr..

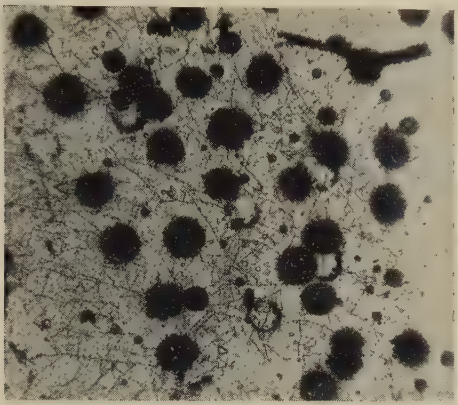


Fig. 7. *Allothyriopsis landolphiae* Bat. & Maia n. sp. Picnostromas e micelio.

radiato, ex cellulis rectangularibus, 4—5 × 3—4μ, marginibus fimbriatis ex cellulis 12.5—19 × 1—2 μ; pariete basali inconspicuo. Hymenium inversum. Pycnidiosporae sessiles, cylindraceo-fusoideae, 3-transversliter septatae, constrictae, primo hyalinae dein olivaceae, 19—20 × 2.5—3 μ.

In foliis *Landolphiae owariensis* socia *Asterinellae sp.* Aburri, Ghana, S. J. Hughes, 24.5.947. Typus, 875, Ghana Myc. Herb. et 44410, Comm. Myc. Inst. Lond.

Thyriostromella Bat. & Costa n. gen.
Tipo: *T. calophyllii* n. sp.

Micélio superficial formado por hifas reticuladas, marron, não setoso e sem hifopodios. Picnidios superficiais, escutelares-dimidiados, orbiculares, marron, astomos, de dehiscência estelar, prosenquimático-radiados, glabros, uniloculares. Himenio invertido; conidioforos nodulares, paliçadicamente dispostos. Picnidiosporos alongados, 1-septados, lisos, multigutulados, hialinos.

Mycelium superficiale ex hyphis reticulatis, brunneis, non setosis, sine hyphopodiis, compositum. Pycnidia superficialia, scutato-dimidiata, orbiculata, brunnea, astoma, stellata dehiscentia, prosenchymatico-radiata, glabrata, uniloculata. Hymenium inversum; conidiophoris nodularibus, paliçadicis, compositis. Pycnidiosporae elongatae, 1-septatae, leves, pluriguttulatae, hyalinae.

Mindoa Petrak Died. offiuis, sed pycnidia stellatim dehiscentia cum pycnidiosporis 1-septatis, acrogenis in conidiophoris nodulatis, paliçadicis composita (vide Ann. Mycol. XI: 176, 1913).

Thyriostromella calophyllii (SACC.) BATISTA & COSTA n. comb.
Syn.: *Leptothyrella calophyllii* SACC.

Anfigeno. Micélio superficial, recobrindo aos picnidios, de modo escasso, composto por hifas marron, reticuladas, septadas, pouco constrictas, com células de 8—16.5 × 3—5.5 μ, não setoso e sem hifopodios. Picnidios superficiais, visiveis sob o micélio, isolados ou gregários, escutelares, dimidiados, obiculares, 195—270 μ de diam, 24.5—31 μ de alt, uniloculares, glabros, de dehiscência estelar: parede superior membranacea, prosenquimática-radiada, 9—15.5 μ de espessura, constituida por celulas cilindraceas de 5—13.5 ×

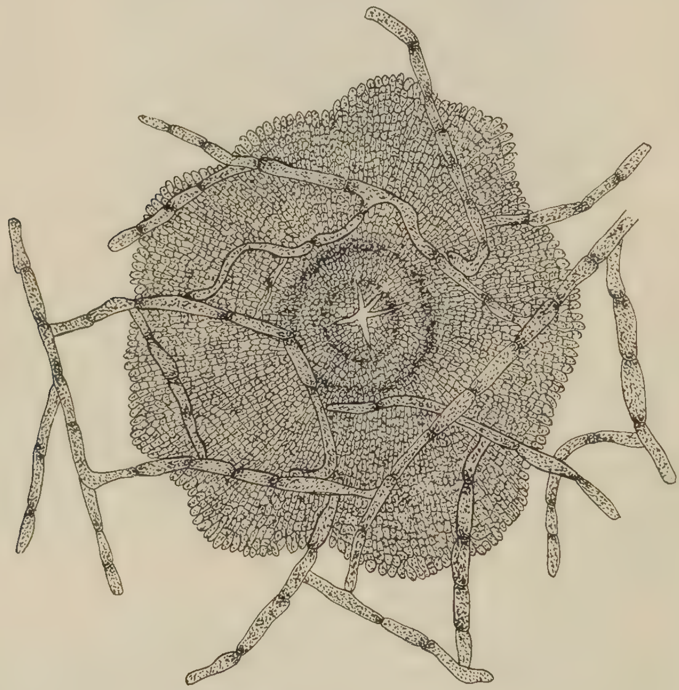


Fig. 8. *Thyriostromella calophylli* BAT. & COSTA n. sp. Picnidio e micélio reticulado. Orig.

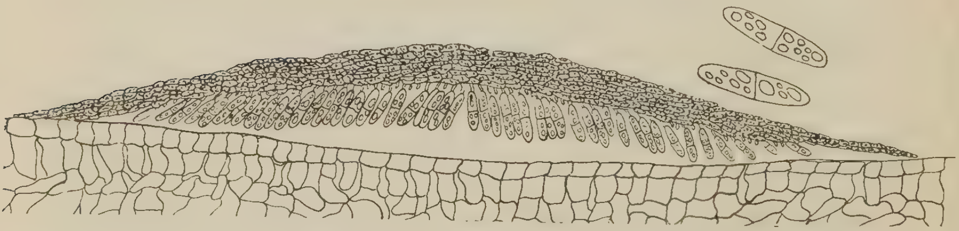


Fig. 9. *Thyriostromella calophylli* BAT. & COSTA n. sp. Picnostroma e picnidiosporos. Orig.

3.5—4 μ; parede inferior inobservada. Himenio invertido; conidioforos nodulares, paliçadicos, hialinos, 1—1.5 μ de diam, muito curtos. Picnidiosporos elipsoides, 1-septados, não constrictos, multigutulados, hialinios lisos, 22—27 × 5—7 μ.

Sôbre folhas de *Calophyllum floribundum* — Singapura, Straits Settlements, Sept. 1927. Tipo, nº 445, Magyar Nemzetti Museum — Budapest.

Amphigena. Mycelium superficiale pycnidiis conspicuit inductum ex hyphis brunneis, reticulatis, septatis, parvum constrictis, ex cellulis 8—16.5 × 3—5.5 μ efformatis, non setosum nec hyphopodiatum, compositum. Pycnidia superficialia, sparsa vel gregaria dimidiato-scutata, orbiculata, 195—270 μ diam., 24.5—31 μ alt., uniloculata, glabra, stellata dehiscentia; pariete basali non viso; pariete superiore membranoso, prosenchymatico-radiato, 9—15.5 μ cr., ex cellulis cylindraceis, 5—13.5 × 3.5—4 μ, efformato. Hymenium inversum. Conidiophoris nodularibus, parallele-stipatis, 1—1.5 diam. Pycnidiosporae ellipsoideae, 1-septatae, non constrictae, leves, pluriguttulatae, hyalinae, 22—27 × 5—7 μ.

In foliis *Calophylli floribundum* — Singapore. Straits Settlements, Sept. 1927. Typus, nº 445, Magyar Nemzetti Museum — Budapest.

Obs.: Graças á colaboração de Dr. BOHUS GÁBÓR, logramos examinar êsse especimen do Magyar Nemzetti Museum, Budapest, o qual tem a seguinte etiqueta: ,,Fungi Malayana, C. F. BAKER, nº 445, *Leptothyrella calophylli* SACC. n. sp., on *Calophyllum floribundum*, Island of Singapore, Sept. 27, Det. Saccardo.''

Trata-se assim da espécie tipo de SACCARDO que ora elegemos como tipo do novo gênero **Tyriostromella**, por seus picnidios de himenio invertido. *Consultar*: SACCARDO, *in* Syll. Fung. XXV: 514, 1931; DIEDICKE, *in* Ann. Mycol. XI: 176, 1913. h/

Leprieurina winteriana ARNAUD

in Ann. École Nat. Agric. Montp. XVI: 211, 1918 A. PUTTEMANS, Fungi S. Paulenses, nº 149 — sob *Asterina anonicola* P. HENN. n. sp. Hedwigia, 1902, pag. 107.

Sin.: *L. goyazensis* (P. HENN.) ARNAUD. (loc. cit.).
Asterula? goyazensis (P. HENN.) ULE, nov. '92 (Herb. Maubl.).

Plágulas marron-negras, epifilas, superficiais, tenues, circulares, isoladas ou confluentes, 2—8 mm de diam. Micélio marron, superficial, septado, não setoso, não hifopodiado, radiado-reticulado, de hifas retilineas, pouco flexuosas, formadas de células de 15—40 × 6—7.5 μ; ramificações opostas ou unilaterais em angulos de 45 a 90°. Picnostromas escutelares, marron-negros, plano-convexos, glabros, isolados ou confluentes, ástomos, de dehiscência irregularmente estelar 160—220 μ de diam.; parede superior radiada de células retangulares de 5.5—9 × 2.5—5.5 μ; bordos franjados com hifas que se estendem de 10—25 μ. Himenio invertido; parede basal tenue, marron-clara. Haustorios globosos, reniformes ou elipsoides,

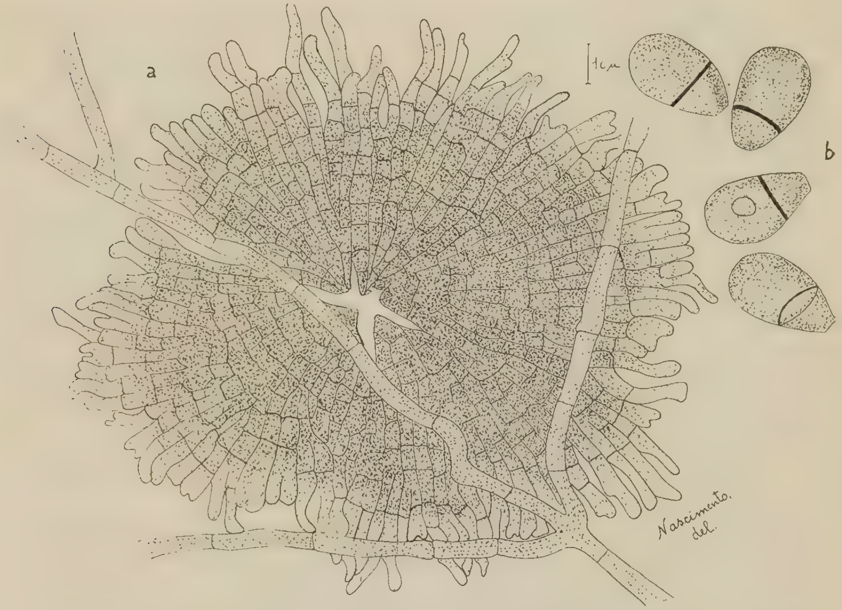


Fig. 10. *Leprieurina winteriana* Arnaud.
a) picnostroma e micelio; b) picnidiosporos. Orig.

9.5—14 μ de diam., continuos, marron, subcuticulares a epidermais, originando prolongamentos tambem continuos, mas filiformes no sentido da parede superior dos picnostromas. Conidioforos obsoletos ou ausentes. Picnidiosporos claviformes ou piriformes, 1-septados, de celulas desiguais, conoide e pequena e inferior e subglobosa e volumosa a superior, não constrictos, numerosos, marron-negros, 20.5—30 × 14.5—19.5 μ.

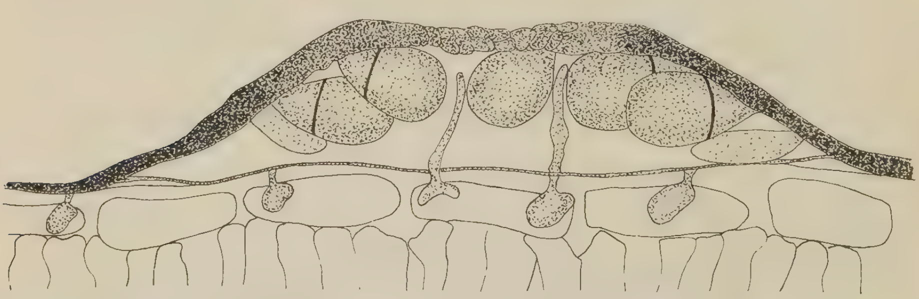


Fig. 11. *Leprieurina winteriana* Arnaud.
Picnostroma sob córte longitudinal, observando-se os haustorios com os seus prolongamentos no seio da cavidade picnostromatica. Orig.

Sôbre folhas de *Anona sp.* associado a *Echidnodella guatemalensis* Bat. × Nasc., *Phaeodimeriella parvula* (Cke) Hansf. var. *echidno-*

dellae Bat. & Nasc., *Helminthosporium* e *Catenularia*. Dept. Santa Rosa, perto de El Molino, Guatemala, Leg. P. C. Standley, Nov. 26, 1940. Espec. nº 78349, The National Fungus Collections e nº 11000, no Instituto de Micología, da Universidade do Recife. Det. A. Chaves Batista & M. Lourdes Nascimento.

NOTA: Agradecemos ao Dr. John A. Stevenson o envio dêsse material de *Anona sp.* sôbre o qual assinalamos essa especie de *Leprieurina*.

Septothyrella bactridis Bat. & Ciferri n. sp.

Picnostromas epifilos, superficiais, espalhados, marron-claros, orbiculares, 90—135 μ de diam, plano-escutelares, ostiolados, glabros; parede superior membranosa, radiada, 5—12 μ de espessura, formada por células retangulares ou quadráticas, 2.5—3.5 × 2—3,5 μ; margens franjadas, onde se destacam hifas alongadas de 1.5—2 μ de diam. Himenio invertido. Conidioforos denticulares, hialinos, quasi obsoletos. Picnidiosporos clavados ou elipsoides, 1—3-septádos, gutulados, não constrictos, hialinos, 7—9,5 × 2.5 — 3.5 μ. Parede basal inconspicua.

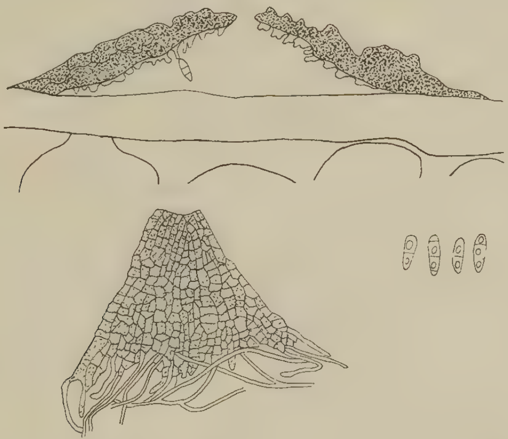


Fig. 12. *Septothyrella bactridis* Bat. & Ciferri n. sp.
a) Picnostroma em secção longitudinal; b) detalhe da constituição da parede superior do picnostroma; c) picnidiosporos.

Sôbre folhas de *Bactris maraja* M. Dois Irmãos — Recife. Leg. Oswaldo S. da Silva, 15/9/57. Tipo 11869, Instituto de Micologia, Universidade do Recife. Associado a *S. pernambucensis* n. sp. *Meliola sp.*, *Dimerium venturioides* (Sacc. & Berl.) Theiss. *Calonectria erysiphoides* Berl. e *Trichothelium annulatum*.

Pycnostromata epiphylla, superficialia, sparsa, brunnescentia, orbiculata, plano-scutata, 90—135 μ diam., glabrata, ostiolata; pariete superiore membranoso, radiato, 5—12 μ cr., ex cellulis rectangularibus vel quadratis, 2.5—3.5 × 2—3.5 μ efformato,

marginibus fimbriatis, ex hyphis elongatis, 1.5—2 μ cr. compositis; pariete inferiore inconspicuo. Conidiophoris denticulatis vel obsoletis. Pycnidiosporae clavatae vel ellipsoideae, 1—3-septatae, guttulatae haud constrictae, hyalinae, 7—9.5 × 2.5—3.5 μ.

In foliis *Bactris marajae* — Dois Irmãos — Recife. Leg. Oswaldo S. da Silva, 15/9/57. Typus, 11869, Instituto de Micologia, Universidade do Recife. Socia *Meliolae sp.*, *Dimerium venturioides* (Sacc. & Berl.) Theiss. *Calonectriae erysiphoides* Berl. *Septothyrellae pernambucensis* n. sp. et *Trichothelii annulati*. (Karst.) R. Sant.

Septothyrella nipponica Hino & Katumoto
in Bull. Fac. Agric. Yamaguti Univ. Japan. nº 6: 31, 1955.

Plagulas superficiais, efusas, membranaceas, fuligineas. Picnostromas gregarios, escutelares, 300—500 μ de diam., membranaceos, fuligineos, não radiados, de margens indistintas; ostiolo de 50—60 μ de diam. Himenio invertido. Conidioforos quase obsoletos. Picnidiosporos fusoides, 3-septados, pouco constrictos, hialinos, gutulados, 18.5—28 × 4.5—6 μ.

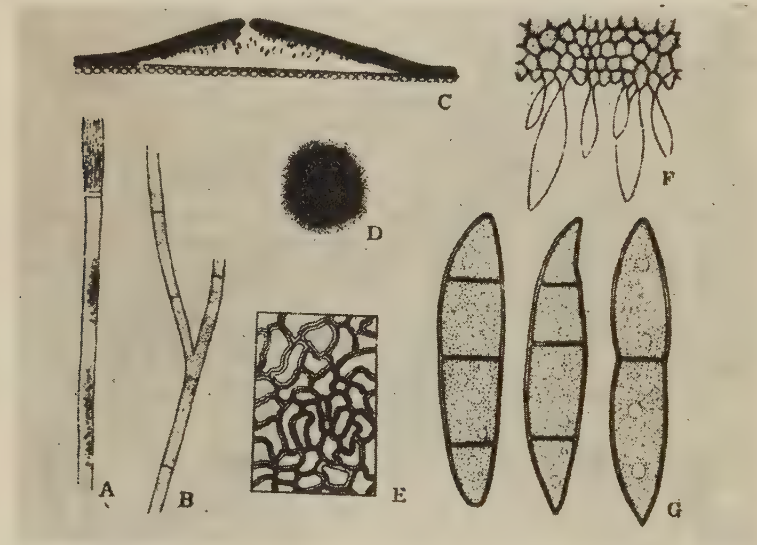


Fig. 13. *Septothyrella nipponica* Hino & Katumoto
a) Lesões do colmo; b) Hifa do micélio; c) Picnostroma sob secção transversal; d) Picnostroma sob vista frontal; e) Contexto do picnostroma; f) Conidioforos e picnidiosporos jovens; g) Picnidiosporos.

Extr. do Bull. Fac. Agric. Yamaguti. Univ. Japan, nº 6: 31, 1955.

Sobre colmos de *Pleioblastus simoniozuki* — Nagato, Japão — Leg. N. Miake, 25/4/955.

Septothyrella pernambucensis Bat. & Ciferri n. sp.

Picnostromas anfígenos, superficiais, espalhados, orbiculares, plano-escutelares, 110—205 μ de diam, marron, glabros, ostiolados, membranosos; parede superior 4—10 μ de espessura, radiada, com células retangulares de 11—13.5 × 5—8.5 μ, margens crenuladas, parede basal inconspicua. Himenio invertido. Conidioforos denticulados, marron. Picnidiosporos clavado-caudados, 0—3-septados, gutulados ou não, hialinos, 28—38 × 4—4.5 μ.

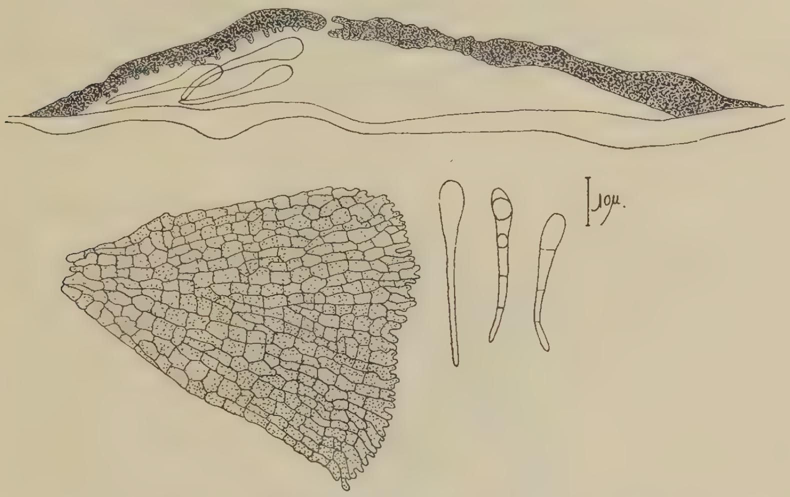


Fig. 14. *Septothyrella pernambucensis* Bat. n. sp.
a) picnostroma sob secção longitudinal; b) sector da parede superior do picnostroma; c) picnidiosporos.

Sôbre folhas de *Bactris maraja* M. — Dois Irmãos. Recife. Leg. Oswaldo S. da Silva 15/9/57. Tipo 11870, Instituto de Micologia, Universidade do Recife — Associado a *S. bactridis* n. sp., *Meliola sp.*, *Dimerium venturioides* (Sacc. & Berl.) Theiss., *Calonectria erysiphoides* Berl. e *Trichothelium annulatum* (Karst.) R. Sant.

Pycnostromata amphigena, superficialia, sparsa, orbiculata, plano-scutata, 110—205 μ diam., brunnea, glabrata, ostiolata, membranosa; pariete superiore 4—10 μ cr. radiato, ex cellulis rectangularibus, 11—13.5 × 5—8.5 μ efformato, marginibus crenulatis; pariete basali inconspicuo. Hymenio inverso. Conidiophoris denticulatis, brunneis, minutis. Pycnidiosporae clavatae, caudatae, 0—3-septatae, guttulatae vel non, hyalinae 28—38 × 4—4.5 μ.

In foliis *Bactridis marajae* — Dois Irmãos — Recife. Leg. Oswaldo S. da Silva, 15/9/57. Typus, 11870, Instituto de Micologia, Universidade do Recife. Socia *S. bactridis* n. sp. *Meliolae sp.*, *Dimerii venturioides* (Sacc. & Berl.) Theiss., *Calonectria erysiphoides* Berl. et *Trichothelii annulati* (Karst.) R. Sant.

Asterostomula Theiss.
in Ann. Myc. XIV: 270, 1916.
Tipo: *A. loranthi* Theiss.

Micélio superficial, marron, de hifas septadas, sem hifopodios ou setas. Picnostromas superficiais, dimidiado-escutelares, dehiscencia estelar-irregular, radiados, marron; himenio invertido. Conidioforos muito curtos ou obsoletos. Picnidiosporos continuos, marron, ovoides, piriformes ou oblongos.

ASTEROSTOMULA — Espécies aceitas

Chave baseada no tamanho dos picnostromas e dos picnidiosporos.

A. patula Petrak *in* Sydowia, IV: 549, 1950	40—80 μ diam	15—23 × 10—18 μ
A. lepidotricha Theiss. *in* Ann. Myc. XIV: 271, 1916 . . .	55—70 μ diam	19—23 × 12—14 μ
A. pelladensis (P. Henn.) Bat. & Vital *in* Hedwigia 48:15, 1908	60—80 μ diam	15—20 × 10—13 μ
A. loranthi Theiss. *in* Ann. Myc. XIV: 270, 1916 . . .	60—85 μ diam	25—28 × 16—18 μ
A. pinatubensis Syd. *in* Leaf Parasit. Fung. IX : 3177, 1934	50—90 μ diam	17—22 × 11—14 μ
A. premnae Bat. & Nasc. . .	55—105 μ diam	18—20 × 14.5—16 μ
A. pseudospondiadis Bat. & Maia	85—110 μ diam	21.5—37 × 10—19 μ
A. puyana Petrak *in* Sydowia, IV : 450, 1950	80—150 μ diam	15—39 × 8—12 μ
A. caperoniae (Speg.) Bat. & Cif. *in* Mycet. Arg. V, pag. 396, 1910	90—100 μ diam	18—22 × 10—12 μ
A. subreticulata (Speg.) Bat. & Cif. *in* Fung. Guar. nº. 143	100—150 μ diam	20—24 × 14—16 μ
A. subreticulata var. *microspora* (Speg) Bat. & Cif. *in* Fung. Guar.	100—150 μ diam	16—20 × 10—12 μ

Asterostomula caperoniae (Speg) Bat. & Ciferri n. comb.
Sin.: *Asterostomella caperoniae* Speg.
in Mycet. Arg. V, pag. 396, 1910.

Plágulas orbiculares, pequeninas, porém confluentes, 1—10 mm de diam, negras. Micélio de hifas septadas, de ramificações alternas, 3—4 μ de diam, fusco-olivaceas, sem hifopodios. Picnostromas orbiculares, fuligineos, 90—100 μ de diam, de dehiscência estelar e margens fimbriadas. Picnidiosporos obtusos, 1—2-gutulados, lisos, marron, 18—22 × 10—12 μ. Conidioforos subhialinos, conoides.

Sôbre folhas de *Caperonia palustris*, Bompland, Missiones, Out. 1909, Leg. P. Jorgensen.

Obs.: As setas a que alude Spegazzini são conidoforos de um Dematiaceae associado.

Est *Asterostomula* cum mycelio non hyphopodiato.

Asterostomula pelladensis (P. Henn.) Bat. & Vital n. comb.
Sin.: *Asterostomella pelladensis* P. Henn.
in Hedwigia, 48: 15, 1908.

Plágulas hipofilas, localizadas na zonas mediana da folha, ao longo da nervura principal, tenues, negras, de fórma irregular, alcançando até 18 mm na maior dimensão. Micélio pouco abundante, de hifas marron-claras, 2—3 μ de diam., de septação pouco evidente, sem hifopodio. Picnostromas escutelares arredondados, 40—80 μ de diam. numerosos, marron, radiados, de fenda estelar, membranosos, parede superior transparente, deixando ver os picnidiosporos. Himenio invertido; parede basal inconspicua. Conidioforos quase obsoletos. Picnidiosporos elipsoides ou ovoides, 12.5—20 × 9.5—13 μ, marron-escuros continuos.

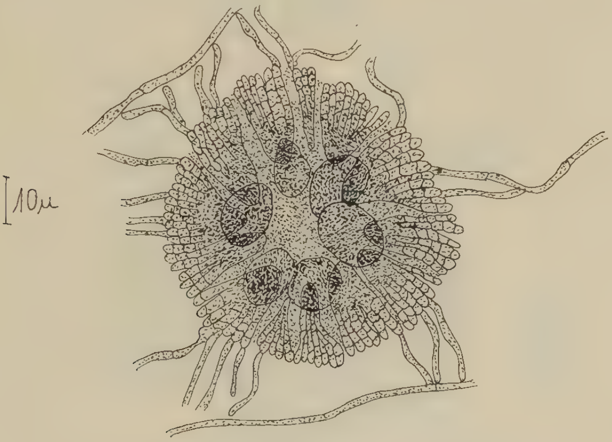


Fig. 15. *Asterostomula pelladensis* (P. Henn.) Batista & Vital n. comb. Picnostroma e hifas do micélio. Orig.

Associado a *Hysterostoma microspora* sôbre *Cassipourea gerrardi* — Woodbush, Zonthausberg, Transvaal, Leg. E. M. Doidge, 16.9.923. Lectotipo n⁰ 17781 em Union of South Africa, Dep. Agric. Mycol. Herb. Det. A. Chaves Batista & A. F. Vital, 26.5.957.

Est *Asterostomula* cum mycelio non hyphopodiato.

Asterostomula pinatubensis Syd.
in Leaflets of Philippine Botany, IX: 3177, 1934.

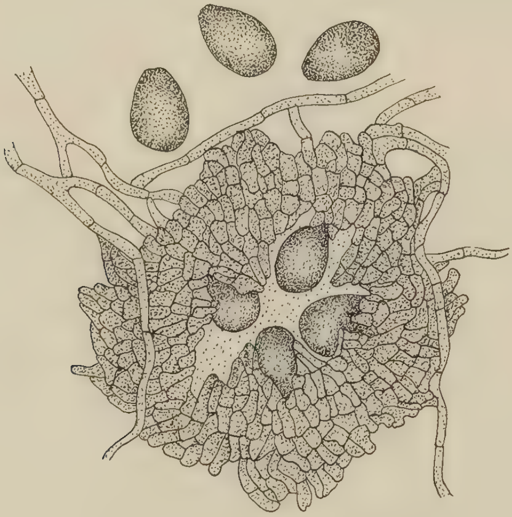
Plágulas hipofilas, isoladas ou confluentes, irregulares, 1—5 mm de diam, cinza-negras. Micélio de hifas intercruzadas, pouco ramificadas, onduladas, septadas, 2.5—3.5 μ de diam., não hifopodiadas e sem setas. Picnostromas orbiculares, 50—90 μ de diam, de dehiscência estelar, marron, radiados, de margens fimbriadas; himenio

invertido. Picnidiosporos ovoides, ovoide-oblongos ou piriformes, continuos, marron, com uma gútula central, 17—22 × 11—14 μ.
Sôbre folhas de *Ilex brunnea* Merr. (nº 21957 — Ehner).

Obs.: Não examinamos êsse especimen.

Asterostomula premnae Bat. & Nasc. n. sp.

Micélio comum a *Asterinella creberrima* Syd. Picnostromas superficiais, orbiculares, 55—105 μ de diam, 35—45 μ de alt., dispersos. ou confluentes, marron-negras, radiados, de margens franjadas, a parede superior formada por células retangulares, 3.5—9 × 2—5 μ, membranosos, de dehiscência estelar-irregular. Himenio invertido.



Nascimento, del.

Fig. 16. *Asterostomula premnae* Bat. & Nasc. n. sp. Picnostroma e picnidiosporos. Orig.

Parede basal indistinta, Picnidiosporos sesseis, ovoide-globosos, continuos, marron, 18—20 × 14.5—16 μ, numerosos.

Sôbre folhas de *Prenna sp.* associado a *Asterinella creberrima* Syd. Los Baños, Province Laguna, Luzón, Philippines. Leg. C. F. Baker, Jan. 1914. Tipo, nº 512, Magyar Nemzetti Museum — Hungria.

Mycelium ut sub *Asterinella creberrima* Syd. Pycnostromata superficialia, orbiculata, 55—105 μ diam., 35—45 μ alt., sparsa vel confluentia, atro-brunnea, radiata, marginibus fimbriatis; pariete superiore membranoso, ex cellulis rectangularibus, 3.5—9 × 2—5 μ efformato, stellato-irregulariter dehiscentibus, hymenium inversum; pariete basali inconspicuo. Pycnidiosporae sessiles, ovoide-globosae, continuae, brunneae, 18—20 × 14.5—16 μ, numerosae. In foliis *Premnae sp.* socia *Asterinellis creberrimae* SYD. Los Baños, Prov. Laguna, Luzon, Philippines, Leg. C. F. BAKER, Jan. 1914. Typus, nº 512, Magyar Nemzetti Museum.

Obs.: SYDOW (Ann. Myc. XV: 247, 1917) apenas notara a presença desses picnidiosporos, chamando-os, simplesmente, ,,conidia ovata'', etc.).

Asterostomula pseudospondiadis BATISTA & MAIA n. sp.

Micélio o mesmo de *Asterinella pseudospondiadis* HANSF. (*in* Proc. Linn. Soc. Lond., 157 sess. part 1, pag. 30, 1944—1945). Picnostromas escutelares, orbiculares, radiados, marron-negros, 85—110 μ de diam., irregularmente espalhados, parede superior membranosa formada por células poligonais ou retangulares, radialmente dispostas, 3.5—5 μ diam.; bordos fimbriados, com hifas até 60 μ de estensão, e células de 13.5—32 × 2.5—3 μ; dehiscência estelar-irregular; himênio invertido; parede basal hialina, inconspícua. Picnidiosporos sésseis, marron-escuros, elipsoides, lisos,

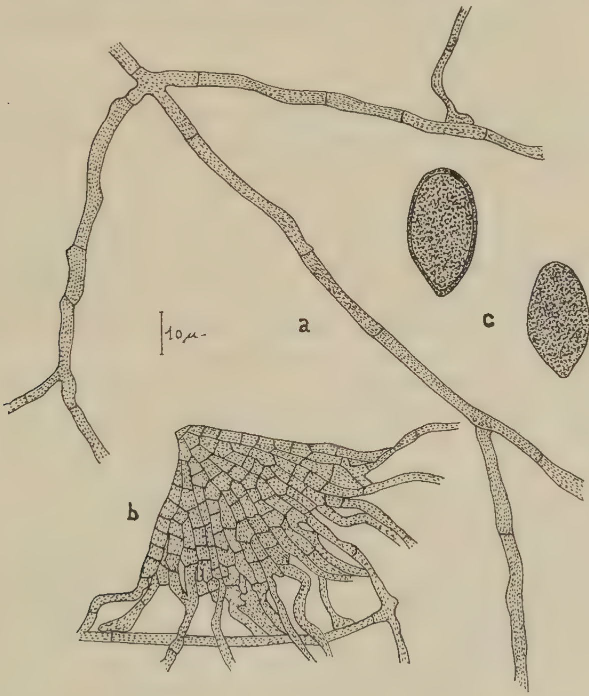


Fig. 17. *Asterostomula pseudospondiadis* BATISTA & MAIA n. sp. a) sector da parede superior de picnostroma; *b*) hifas do micelio; c) picnidiosporos.

unicelulares, 21.5—37 × 10—19 μ, com 2 polos equatoriais, de germinação. Ascostromas de *Asterinella pseudospondiadis* imaturos.

Sôbre folhas de *Pseudospondias microcarpa*, Tafo, Ghana Col. S. J. HUGHES, 16.6.949. Herb. Com. Myc. Inst. 41554 (b). Tipo nº 635 (b) no Ghana Myc. Herb.

Mycelium ut sub *Asterinella pseudospondiadi* HANSF. Pycnostromata scutata, orbiculata, radiata, atro-brunnea, 85—110 μ diam., sparsa; pariete superiore membranaceo, ex cellulis polygonalibus et rectangularibus, 3.5—5 μ diam efformato; marginibus fimbriatis, ex hyphis usque 60 μ longis, ex cellulis 13.5—32 × 2.5—3 μ efformatis; stellatim vel irregulariter dehiscens; hymenium inversum; pariete basali inconspicuo. Pycnidiosporae sessiles, brunneae, ellipsoideae, continuae, leves, 21.5—37 × 10—19 μ. — In foliis *Pseudospondiadis microcarpae*, Tafo, Ghana Leg. S. J. HUGHES, 16.6.949. Typus 635, Ghana Herb. Mycol.

Obs.: Os picnostromas dêsse fungo foram previamente descritos por HANSFORD (loc. cit.).

Asterostomula subreticulata (SPEG.) BAT. & CIFERRI, n. comb.
Sin.: *Asterostomella subreticulata* SPEG.
in Fung. Guar. nonn. nº 143.

Plágulas epifilas, tenues, dispersas ou confluentes, aracnoides, negras. Micélio de hifas alterno-ramificadas, marron, 3—4 μ de diam, sem hifopodios. Picnostromas escutelar dimidiados, marron-negros, 100—150 μ de diam, radiantes, estelar dehiscentes, de margens crenulado-fimbriadas. Picnidiosporos continuos, piriformes ovoides, 20—24 × 14—16 μ, marron-negros.

Sôbre folhas de *Cestrum pubescens* — Guarapi e Vila Rica, Brasil.

Quod. mycelio non hyphopodia *Asterostomula* to.

Asterostomula subreticulata var. *Microspora* (SPEG.) n. comb.
Sin.: *Asterostomella subreticulata* var. *microspora* SPEG.
in Fung. Guar. nonn. nº 143.

As caracteristicas do micelio e dos picnostromas são iguais ás de *Asterostomula subreticulata*.

Os picnidiosporos porém, são menores, de 16—20 × 10—12 μ.

Sôbre folhas de *Cestrum*.

Sed mycelio non hyphopodia to ut *Asterostomula*. A typo differt picnidiosporis minoribus.

Asterinothyriaceae BAT., CIFERRI & MAIA n. fam.

Micélio livre hifopodiado. Picnostromas escutelares, radiados, marron-negros, com hipostroma ou não. Himênio invertido. Conidióforos presentes. Picnidiosporos vários.

Tipo: **Asterinothyrium** BAT., CIFERRI & MAIA.

Mycelium liberum, hyphopodiatum. Pycnostromata scutata, radiata, atrobrunnea, cum hypostromatis vel non. Hymenium inversum. Conidiophoris presentibus. Pycnidiosporae variae.
Typus: **Asterinothyrium** BAT., CIFERRI & MAIA.

Esta família reune as fórmas imperfeitas associadas aos Asterinaceae.

ASTERINOTHYRIACEAE
CHAVE PARA OS GENÊROS

Micelio livre hifopodiado.

HIALOAMEROSPOROS

A) Picnostromas radiados.
B) Sem hipostroma; dehiscencia irregular; conidioforos curtos.
C) Picnidiosporos acrogenos; ostiolo definido .... **Peltasteropsis** BAT. & MAIA
CC) Picnidiosporos acrogenos .. **Peltasterella** BAT. & MAIA
BB) Hipostroma presente; ostiolo definido; conidioforos curtos; picnidiosporos acrogenos **Asterinothyrium** BAT. & MAIA

FEOAMEROSPOROS

A) Picnostromas radiados; dehiscencia estelar.
B) Sem hipostroma .... **Asterostomella** SPEG. (Fungi Guaranit. 1: 151)
BB) Hipostroma presente . **Asterostomopsis** BAT. & MAIA

HIALODIDIMOSPOROS

Hipostroma presente; picnostromas radiados; dehiscencia irregular; picnidiosporos sesseis ..... **Rota** BAT. & CIF.

Asterinothyrium BATISTA, CIFERRI & MAIA, n. gen.
Tipo: *Asterinothyrium singulatum* nobis.

Micélio livre, hifopodiado, não setoso, marron, de hifas radiantes, anastomosantes, septadas. Picnostromas superficiais, orbiculares, ostiolados, não setosos. Parede superior radiada. Hipostroma subcuticular. Conidióforos curtos. Picnidiosporos acrógenos, unicelulares, bacilares a cilindráceos, hialinos.

Mycelium superficiale, hyphopodiatum, non setosum, brunneum, ex hyphis radiantibus, anastomosantibus, septatis, compositum. Pycnostromata orbiculata, ostiolata, glabra. Pariete superiore radianti. Hypostromata subcuticularia. Conidiophoris minutis. Pycnidiosporae continuae, acrogenae, bacillares vel cylindraceae, hyalinae.
Typus: *A. singulatum*, nobis.

Asterinothyrium singulatum BATISTA & MAIA, n. sp.

Micélio epífilo, marron-negro, superficial, de hifas tortuosas, anastomosantes, ramificadas em ângulos de 45°, alterna ou unilateralmente septadas, com células de 14—27 × 3.5—6.5 μ. Setas ausentes. Hifopódios laterais, nodulares a sub-globosos, alternos ou unilateralmente dispostos, retos ou recurvados, 8—9.5 μ de diam. Picnostromas orbiculares, isolados ou confluentes, marron-negros, 48—50 μ de diam., 10—12 μ de alt. Ostíolo pouco distinto, 2.5—3 μ diam. Parede superior formada por células quase indistintas, radialmente dispostas, 5—8 μ de espessura. Parede inferior inconspicua; hipostroma sub-cuticular. Himênio invertido. Conidióforos filiformes, hialinos, simples, 2.5—7 × 2.5—3 μ. Picnidiosporos bacilares a cilindráceos, hialinos, lisos, unicelulares, 2.7—6.5 × 2.5—3 μ.

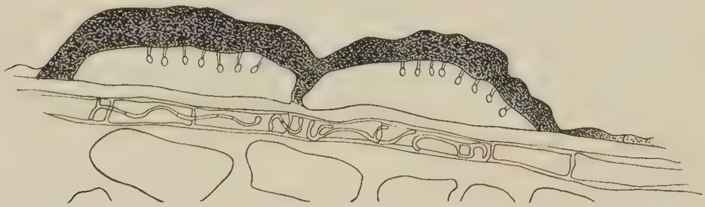


Fig. 18. *Asterinothyrium singulatum* BATISTA & MAIA n. sp. Picnostroma sob córte longitudinal, notando-se a presença de hipostroma sub-cuticular.

Sôbre folhas de *Trichocladus ellipticus* associado a *Isipinga contorta* DOIDGE. Pirie Forest, Cape. Union of South Africa. Pretoria. Leg. E. M. DOIDGE, 18.7.1927. Tipo nº 22427 do Myc. Herb. do Dep. Agr. da Union of South Africa, o qual nos foi gentilmente cedido para exame pelo Dr. P. H. TALBOT.

Mycelium superficiale, epiphyllum, atro-brunneum, ex hyphis tortuosis, alternatim vel unilateraliter ramosis in angulis 45°, ex cellulis 14—27 × 3.5—6.5 μ, non setosis, compositum. Hyphopodia lateraliter, nodulata vel subglobosa, alternata vel unilateraliter disposita, continua, 8—9.5 μ diam. Pycnostromata orbiculata libera vel confluentia, atro-brunnea, 48—50 μ diam., 10—12 μ alt., ostiolo 2.5—3 μ diam.; pariete superiore 5—8 μ cr., ex cellulis indistinctis et radiis dispositis efformato; pariete basali inconspicuo. Hymenium inversum. Conidiophoris filiformibus, simplicibus, hyalinis, 2.5—7 × 2.5—3 μ. Pycnidiosporae cylindraceae vel bacillares, hyalinae, laeves, continuae, 2.7—6.5 × 2.5—3 μ. In foliis *Trichocladi elliptici*, socium *Isipingae contortae* DOIDGE — Pirie Forest. Cape. Leg. E. M. DOIDGE, 18.7.1927. Typus 22427, Myc. Herb. Dep. Agr. Union of South Africa. Pretoria.

Peltasteropsis Batista & Maia n. gen.
Tipo: *P. discretum*, nobis.

Micélio superficial, asterinóide, hifopodiado. Picnostromas superficiais, escutelares, dimidiados, marron-negros, radiados, de dehiscência irregular. Himênio invertido. Conidióforos cilindráceos. Picnidiosporos contínuos, hialinos.

Mycelium superficiale, asterinoide, non setosum, sed hyphopodiatum. Pycnostromata superficialia scutata, dimidiata, atrobrunnea, radiata, irregulariter dehiscentia. Hymenium inversum. Conidiophoris cylindraceis. Pycnidiosporae continuae, hyalinae.

PELTASTEROPSIS — Espécies descritas:

	Picnostromas	Conidioforos	Picnidiosporos
P. alaphiae . . .	30—35 μ	Obsoletos	3—4 × 0,5—1 μ
P. baphiae . . .	35—45 μ	,,	1,5—2,5 × 1—2 μ
P. discretum . .	65—85 μ	2,5—3,5 × 1,5—2 μ	3,5—4 × 1,5—2,5 μ
P. eugeniae . . .	67.5—110 μ	5—6,5 × 2—2,5 μ	10—16,5 × 1,5—2,5 μ
P. jahnii . . .	117—180 μ	Obsoletos	2—4 × 0,6—1,2 μ
P. minutum. . .	40—60 μ	2—3 × 1—1,5 μ	2,5—3 × 1—1,5 μ
P. moquileae . .	80—112 μ	3—5 × 1 μ	2—2,5 × 1—1,5 μ

Peltasteropsis alafiae Bat. & Maia n. sp.

Micélio livre comum a *Asterina blanda* Syd. var. *alafiae* Bat. & Maia. Picnostromas epifilos, orbiculares, dimidiados, marron escuros, 30—35 μ diam; parede superior formada por células retangulares radialmente dispostas, 3—4 × 2.5—3 μ, astomos de dehiscencia em fenda estelar-irregular; parede basal inconspicua.

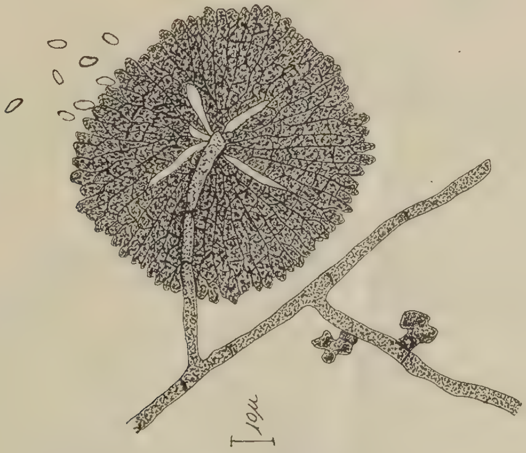


Fig. 19. *Peltasteropsis alafiae* Bat. & Maia n. sp.

Conidioforos obsoletos. Picnidiosporos bacilares a cilindraceos, hialinos, lisos, continuos, 3—4 × 0.5—1 μ. Sôbre folhas de *Alafia scandens*, associado a *Asterina blanda* Syd. var. *alafiae* nobis. Fetish Forest, Pokoasi, Gold Coast. Leg. S. J. Hughes, 1.6.949. Tipo, nº 871, G.C.M. Herb.

Mycelium ut sub *Asterina blanda* var. *alafia*. Pycnostromata epiphylla, orbiculata-dimidiata, brunnea, 30—35 μ diam.; pariete superiore ex cellulis rectangularibus, radialiter dispositis, 3—4 × 2.5—3 μ composito irregulariter disrumpenti; pariete basali inconspicuo. Conidiophoris plerumque obsoletis. Pycnidiosporae bacillares vel cylindraceae, continuae, leves, hyalinae, 3—4 × 0.5—1 μ.

In foliis *Alafiae scandentis*, socia *Asterinae blandae* Syd. var. *alafiae* nobis. Fetish Forest, Pokoasi, Gold Coast. Leg. S. J. Hughes, 1.6.949. Typus, nº 871, in G.C.M. Herb.

Peltasteropsis baphiae Bat. & Maia n. sp.

Micélio comum a *Parasterina baphiae*. Picnostromas epifilos, superficiais, orbiculares, dimidiados, 35—45 μ diam., marron-negros, astomos, de dehiscencia em fenda estelar, com celulas parietais retangulares, 2.5—4.5 × 3—4 μ radialmente dispostas. Bordos não franjados. Himenio invertido; parede basal hialina, tenuíssima. Conidioforos obsoletos. Picnidiosporos bacilares e cilindraceos, lisos, contínuos hialinos 1.5—2.5 × 1—2 μ.

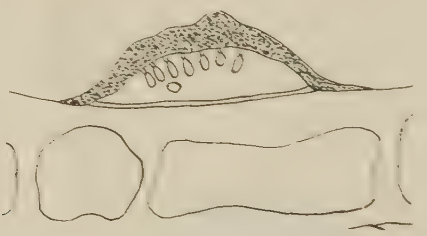


Fig. 20. *Peltasteropsis baphiae* Batista & Maia n. sp. Picnostroma sob corte longitudinal.

Sôbre folhas de *Baphia nitida*, associado a *Parasterina baphiae* (Hughes) Bat. & Maia. Esiama, Ghana S. J. Hughes, 14.5.949. Tipo, nº 32, G.C.M. Herb.

Esta mesma espécie encontramos também noutro especimen de *Baphia nitida*, coletado por S. J. Hughes em Pokoasi, Ghana, 26/4/49, sob o nº 38 do G.C. Myc. Herb.

Mycelium ut sub *Parasterina baphia*. Pycnostromata epiphylla superficialia, orbiculata, dimidiata, 35—45 μ diam., atro-brunnea, stellatim dehiscentia; parietibus ex cellulis rectangularibus; marginibus non fimbriatis; hymenium inversum; pariete basali tenuissimo, hyalino. Comidiophoris obsoletis. Pycnidiosporae bacillares vel cylindraceae, continuae, hyalinae, 1.5—2.5 × 1—2 μ.

In foliis *Baphiae nitidae*, socium *Parasterinae baphiae* (HUGHES) BAT. & MAIA. Esiama, Ghana S. J. HUGHES, 14/5/949. Typus, n⁰ 32, G.C. My. Herb.

Peltasteropsis discretum BATISTA & MAIA, n. sp.

Micélio superficial, asterinóide, de hifas sinuosas, ramificadas irregularmente, reticuladas, septadas, com células de 10.5—60 × 5.5—7 μ, não setoso; hifopódios bicelulares, opostos, alternos ou laterais, 13.5—17.5 × 5.5—7 μ. Picnostromas superficiais, escutelares, dimidiados, radiados, 65—85 μ de diam., marron-negros, com células parietais, irregulares ou retangulares, 5—6 × 3—5 μ; dehiscencia irregular. Himênio invertido. Parede basal indistinta. Conidióforos cilindráceos 2.5—3.5 × 1.5—2 μ, hialinos. Picnidiosporos elípticos ou fusoides, contínuos, hialinos, 3.5—4 × 1.5—2.5 μ.

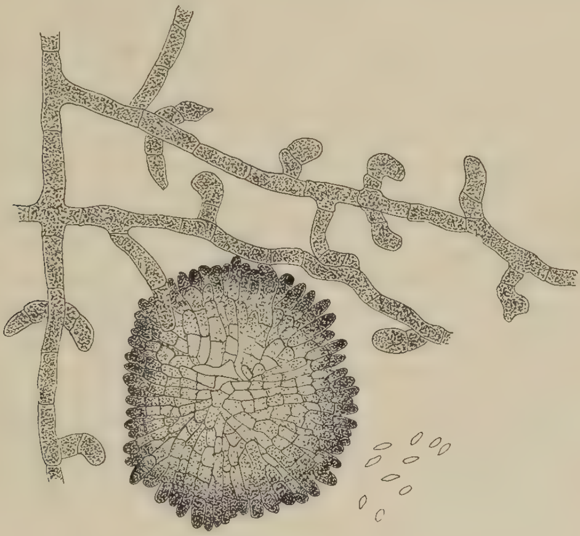


Fig. 21. *Peltasteropsis discretum* BATISTA & MAIA n. sp.
Picnidio, picnidiosporos e micélio hifopodiado. Orig.

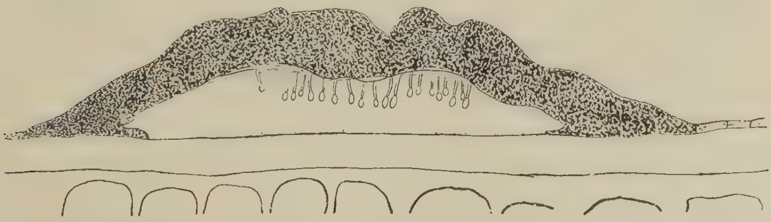


Fig. 22. *Peltasteropsis discretum* BATISTA & MAIA n. sp.
Córte longitudinal de picnidio, mostrando o himenio invertido. Orig.

Sôbre folhas de *Capparis cynophallophora* associado a *Parasterina cynophallophorae* Bat. & Maia e *Irenopsis sp.* São Lourenço, Pernambuco. Leg. A. Chaves Batista, 5.1.56. Tipo 5179, Instituto de Micologia, Universidade do Recife.

Mycelium superficiale, asterinoide, ex hyphis sinuosis, irregulariter ramosis, reticulatis, septatis, ex cellulis 10.5—60 × 5.5—7 μ, non setosis, compositum. Hyphopodia bicellularia, opposita, alternata vel unilateralia, 13.5—17.5 × 5.5—7 μ. Pycnostromatis superficialibus, scutatis, dimidiatis, radiatis, 65—85 μ diam.; pariete superiore ex cellulis irregularibus vel rectangularibus, 5—6×3—5 μ composito; irregulariter dehiscens; pariete basali indistincto. Hymenium inversum. Conidiophoris cylindraceis, continuis, 2.5—3.5 × 1.5—2.5 μ. Pycnidiosporae ellipsoideae vel fusoideae, continuae, hyalinae, 3.5—4 × 1.5—2.5 μ. In foliis *Capparis cynophallophoris* socium *Parasterinae cynophallophorae* et *Irenopsis.* São Lourenço, Pernambuco. Leg. A. Chaves Batista, 5.1.56. Typus 5179, Instituto de Micologia, Universidade do Recife, Prov. Pernambuco, Brasil, Amer. Austr.

Assinalado tambem em associação com *Asterina leonensis* Syd. sôbre *Maosdenia latifolia.* Espec. nº 774, de Ghana Myc. Herb. Tambem associado a *Parasterina concinna* (Syd.) Bat. & Maia, sôbre *Ceropegia* e *Tylophora silvatica* — Ghana, coletado por S. J. Hughes, respectivamente em 13.6.949 e 23.4.949.

Peltasteropsis eugeniae Bat. & Ciferri n. sp.

Plágulas epífilas, marron-negras, membranosas, circulares a subcirculares, isoladas ou confluentes, 1—5 mm de diam. Micélio superficial, marron-escuro, não setoso, telemórfico, septado, não constricto, com ramificações opostas, alternas e unilaterais, regulares, em ângulos de 45—90°; células hifais de 14—27 × 5.5—8 μ. Hifopódios laterais, alternos, cilindráceos a clavados, contínuos, encurvados,

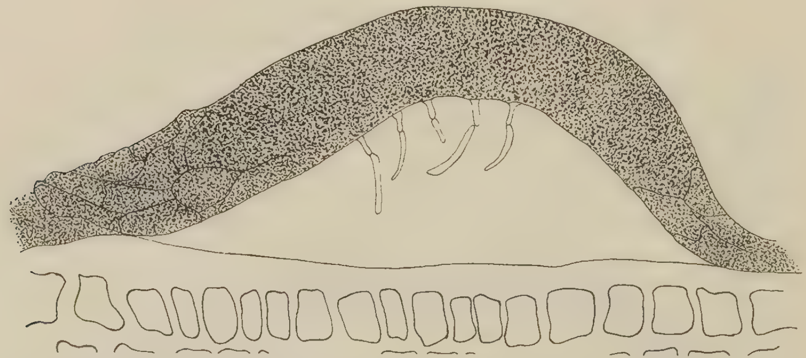


Fig. 23. *Peltasteropsis eugeniae* Bat. & Cif. n. sp.
Picnostroma sob córte longitudinal, observando-se ainda conidiosporos e picnidiosporos.

13—20 × 8—9.5 μ. Picnostromas superficiais, orbiculares, membranosos, marron-oliváceos, isolados, 67.5—110 μ de diam, tendo de 60.5—85 μ de altura, radiados, de dehiscência estelar; parede superior de 30—36.5 μ de espessura, sub-parenquimática, constituida por células retangulares de 6.5—12.5 × 2.5—4 μ. Himênio invertido. Conidióforos contínuos, hialinos, 5—6.5 × 2—2.5 μ. Parede basal inconspícua. Picnidiosporos alantosporos, contínuos, lisos, 10—16.5 × 1.5—2.5 μ.

Sôbre folhas de *Eugenia smithii* POIR. associado a *Parasterina pemphidioides* (CKE.) THEISS. Clyde Mountain, N.S.W. Leg. L. FRASER, VIII, 1934. Tipo nº 45, Dep. of Botany, University of Sydney, New South Wales.

Plagulae epiphyllae, atro-brunneae, membranosae, simplices vel confluentes, rotundatae, 1—5 mm diam. Mycelium superficiale, atro-brunneum, ex hyphis septatis, haud constrictis, oppositis, alternatim vel unilateraliter ramosis in angulis 45—90°, ex cellulis cylindraceis, 14—27 × 5.5—8 μ, compositum. Hyphopodia lateralia, alternata, cylindracea vel clavata, continua, incurvata, 18—20 × 8—9.5 μ. Pycnostromata superficialia, sparsa, orbiculata, olivaceo-brunnea, membranosa, radiata, stellatim dehiscentia; pariete superiore 30—36.5 μ cr. ex cellulis rectangularibus, 6.5—12.5 × 2.5—4 μ composito; pariete basali inconspicuo. Hymenium inversum. Conidiophoris continuis, hyalinis, 5—6.5 × 2—2.5 μ. Pycnidiosporae allantosporae, continuae, leves, hyalinae, 10—16.5 × 1.5—2.5 μ. In foliis *Eugeniae smithii* POIR, socium *Parasterinae pemphidioidis* (CKE.) THEISS. Clyde Mountain, N.S.W. Leg. L. FRASER, 8/934. Typus nº 45, Dep. of Botany, University of Sydney, New South Wales.

Peltasteropsis jahnii BAT. & CIFERRI & MAIA, n. sp.

Micélio epifilo, superficial, marron, de hifas alternadamente ramificadas, com celulas de 18—32 × 4—5 μ, não setoso. Hifopodios laterais, opostos ou somente de um lado das hifas, uni ou bicelulares cilindraceos, 10—17 × 5—10 μ. Picnostromas escutelares, dimidiados, radiados, 117—180 μ de diam, 12.5—15 μ de altura, membranosos, marron-negros, astomos com células parietais de 4—6.5 × 3—4 μ. Himenio invertido; parede basal indistinta. Conidioforos rudimentares. Picnidiosporos bacilares, continuos, hialinos, 2—4 × 0.6—1.2 μ.

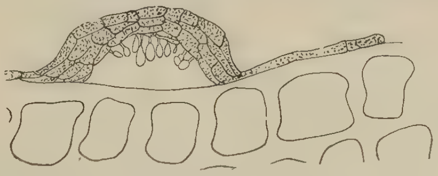


Fig. 24. *Peltasteropsis jahnii* BAT. & MAIA
Córte longitudinal de picnostroma.

Sôbre folhas de *Guatteria sp.* associado a *Asterina jahnii* Syd. (estado imperfeito?) e *Cicinnobella megastoma* Syd. El Limon, Puerto La Cruz — Venezuela, col. H. Sydow, 18.1.928. Tipo 275, no Magyar Nemzetti Museum, Hungria.

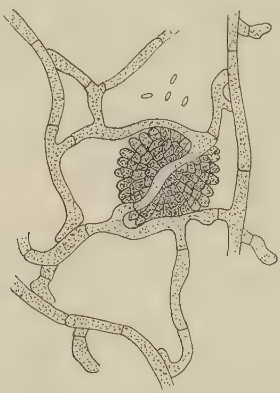


Fig. 25. *Peltasteropsis jahnii* Bat. & Maia
Picnostroma e micélio hifopodiado.

Mycelium epiphyllum, superficiale, brunneum, ex hyphis alternatim ramosis, ex cellulis 18—32 × 4—5 μ, non setosis, compositum. Hyphopodia lateralia, opposita vel non, uni- vel bicellularia, cylindracea, 10—17 × 5—10 μ. Pycnostromata scutata, dimidiata, radiata, 117—180 μ diam., 12.5—15 μ alt., membranosa, atro-brunnea, astoma; pariete superiore unistratosi, ex cellulis 4—6.5 × 3—4 μ efformato. Hymenium inversum. Conidiophoris rudimentaribus. Pycnidiosporae bacillares, continuae, hyalinae, 2—4 × 0.6—1.2 μ. In foliis *Gualterii* sp. socium *Asterinae jahnii* Syd. (status imperfectus?) et *Cicinnobellae megastomae* Syd. El Limon, Puerto La Cruz, Venezuela. Col. H. Sydow, 18.1.928. Typus, Magyar Nemzetti Museum.

Tambem assinalado em associação com *Parasterina hughesii* Bat. & Maia n. sp. Apemdo, Ghana. Leg. S. J. Hughes, 11.5.944. Esp. nº 721, em Ghana Myc. Herb.

Peltasteropsis minutum Bat. & Maia, n. sp.

Micélio superficial, asterinóide, de hifas reticuladas, alterna ou unilateralmente ramificadas, septadas, com células de 21.5—27 × 3—5.5 μ, sem setas. Hifopódios laterais, alternos ou opostos, bicelulares, 11—23 × 5.5—11 μ. Picnostromas superficiais, escutelares, dimidiados, 40—60 μ de diam., radiados, marron-negros, dehiscência irregular; parede superior 2.5—3 μ de espessura, formada por células retangulares de 4.5—6.5 × 3—6 μ; parede basal indistinta. Himênio invertido. Conidióforos filiformes, 2—3 × 1—1.5 μ. Picnidiosporos bacilares, contínuos, hialinos, 2.5—3 × 1—1.5 μ.

Sôbre folhas de *Capparis cynophallophorae* associado a *Parasterina cynophallophorae* var. *longisporae* BAT. & MAIA. Vitória. Leg. R. CIFERRI, 28.3.56. Tipo 5722, Instituto de Micologia, Universidade do Recife.

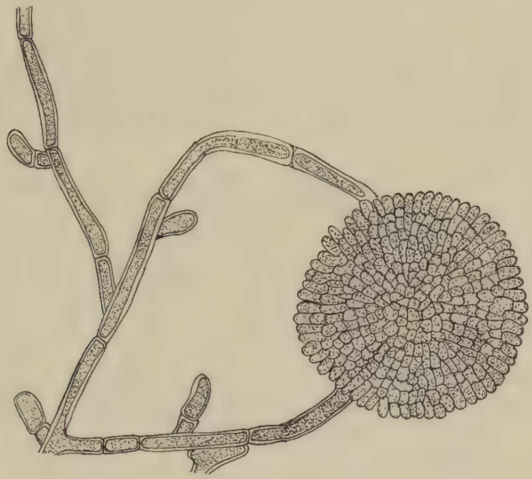


Fig. 26. *Peltasteropsis minutum* BATISTA & MAIA n. sp. Picnidiostroma, hifas do micelio, hifopodiadas. Orig.

Mycelium superficiale, asterinoide, ex hyphis reticulatis, alternatim vel unilateraliter ramosis, septatis, ex cellulis 21.5—27 × 3—5.5 μ, non setosis, compositum. Hyphopodia lateralia, alternata vel opposita, bicellularia, 11—23 × 5.5—11 μ. Pycnostromata superficialia, scutata, dimidiata, 40—60 μ diam., radiata, irregulariter dehiscentia; pariete superiore 2.5—3 μ cr. ex cellulis rectangularibus, 4.5—6.5 × 3—6 μ; pariete basali indistincto.

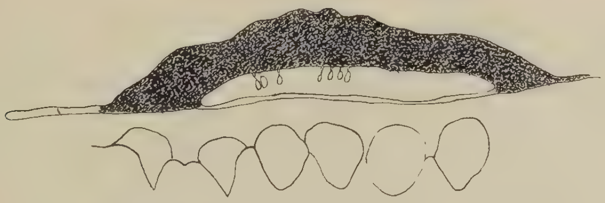


Fig. 27. *Peltasteropsis minutum* BAT. & MAIA.

Hymenium inversum. Conidiophoris filiformibus, hyalinis, 2—3 × 1—1.5 μ. Pycnidiosporae bacillares, continuae, hyalinae, 2.5 — 3 × 1—1.5 μ. — In foliis *Capparis cynophallophoris* socium *Parasterinae cynophallophorae* var. *longisporae* BAT. & MAIA. Vitória. Leg. R. CIFERRI, 28.3.56. Typus 5722, Instituto de Micologia, Universidade do Recife, Prov. Pernambuco, Brasil, Amer. Austr.

Associado tambem a *Parasterina concinna* (Syd) Bat. & Maia, sôbre *Moradenia latifolia* Aburi — Ghana, Leg. S. J. Hughes, 5.5.949. Esp. nº 778 em Ghana Myc. Herb.

Peltasteropsis moquileae Bat. & Maia, n. sp.

Micélio superficial, asterinoide, de hifas marron, retas, ramificadas, alternada, unilateralmente ou de modo oposto, em ângulos de 45—90°, septadas, com células de 17.5—27.5 × 3.5—5 μ. Hifopódios nodulares, intercalares, unicelulares, 10—12.5 × 6—7.5 μ.

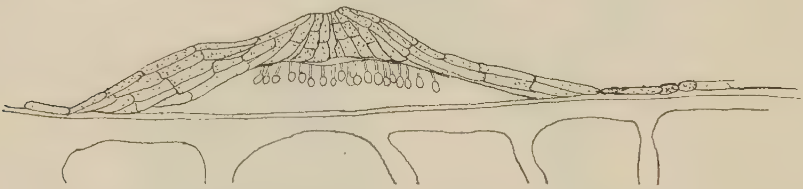


Fig. 28. *Peltasteropsis moquileae* Batista & Maia n. sp. Córte longitudinal de picnidio, observando-se hifopodio, parede superior radiada e himenio invertido. Orig.

Picnostromas superficiais, escutelares, dimidiados, orbiculares, 80—112 μ diam., 18—25 μ de alt., marron, membranosos, glabros, radiados, de dehiscência irregular e margens fimbriadas; parede superior uniestratificada, 8—12 μ de espessura, com células subretangulares, 4.5 × 2 μ; himênio invertido; parede basal indistinta. Conidióforos filiformes, contínuos, hialinos, 3—5 × 1 μ. Picnidiosporos bacilares, contínuos, hialinos, 2—2.5 × 1—1.5 μ.

Sôbre folhas vivas de *Moquileatomentosa* associado a *Cirsosia moquileae* Bat. & Maia e *Asterinopeltis*. Casa Forte, Recife. Leg. Severino José da Silva, 22.4.56. Tipo 5470, Instituto de Micologia, Universidade do Recife.

Mycelium superficiale, asterinoide, ex hyphis brunneis, rectis, septatis, alternatis lateraliter vel oppositis, ramosis, in angulis 45—90°, ex cellulis 17.5—27.5 × 3.5—5 μ, non setosis, compositum. Hyphopodia nodulata, intercalaria, unicellularia, 10—12.5 × 6—7.5 μ. Pycnostromata superficialia, orbiculata, scutato-dimidiata, 80—112 μ diam., 18—25 μ alt., membranosa, glabra, brunnea, radiata, irregulariter dehiscetia, marginibus fimbriatis; pariete superiore unistratoso, 8—12 μ cr., ex cellulis subrectangularibus, 4.5 × 2 μ efformato; pariete basali indistincto. Hymenium inversum. Conidiophoris filiformibus, continuis, hyalinis, 3—5 × 2 μ. Pycnidiosporae bacillares, continuae, hyalinae, 2—2.5 × 1—1.5 μ. — In foliis *Moquilei tomentosae*, socium *Cirsosi moquileae* et *Asterinopeltis*. Casa Forte, Recife. Leg. Severino José da Silva, 22.4.56. Typus 5470, Instituto de Micologia, Universidade do Recife, Prov. Pernambuco, Brasil, Amer. Austr.

Peltasterella Bat. & Maia, n. gen.
Tipo: *P. ocoteae,* nobis.

Micélio superficial asterinoide, hifopodiado, não setoso. Picnostromas superficiais, escutelares, dimiados, radiados, com ostíolo central. Himênio invertido. Conidióforos simples. Picnidiosporos acrógenos, contínuos, hialinos.

Mycelium superficiale, asterinoide, hyphopodiatum, non setosum. Pycnostromata superficialia, scutata, dimidiata, radiata, ostiolata. Hymenium inversum. Pycnidiosporae acrogenae, continuae, hyalinae.

Obs.: Distingue-se de **Peltasteropsis** por seus picnídios de ostíolo central.

PELTASTERELLA — Espécies descritas

	Picnostromas	Conidioforos	Picnidiosporos
P. camaragibeana	90—100 μ diam	1,5—2,5 × 0,5—1 μ	1—1,5 × 0,5—1 μ
P. glochidiae . .	73—95 μ ,,	Até 3 μ compr.	2,5—3 × 1—2 μ
P. lembosicola . .	32,5—50 μ ,,	3—5,5 × 1 μ	3—4 × 0,5—1 μ
P. ocoteae	60—90 μ ,,	3—6 × 1—1,5 μ	3—5,5 × 2,5—3,5 μ
P. piriensis . . .	54—65 μ ,,	3—5 × 0,5—1 μ	1,2—2,5 × 0,5—1 μ
P. spissae. . . .	27—32,5 μ ,,	Obsoletos	1,2—2 × 1,2 μ

Peltasterella camaragibeana Bat. & Maia, n. sp.

Micélio anfígeno, superficial, asterinoide, de hifas reticuladas, marron-negras, septadas, com células de 12.5—20 × 3.5—5 μ, não setoso. Hifopódios mucronados, alternos ou opostos, unicelulares,

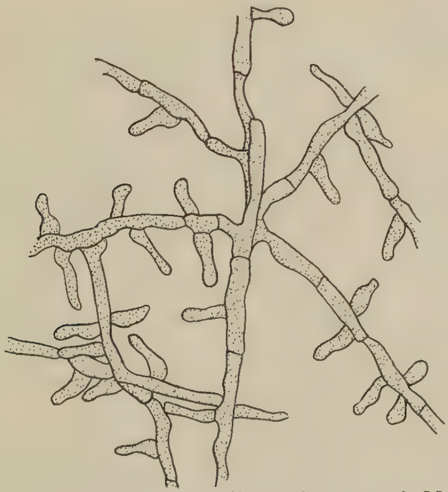


Fig. 29. *Peltasterella camaragibeana* Batista & Maia n. sp. Micélio com hifopódios. Orig.

retos ou não, 12.5—16 × 2.5—4 μ. Picnostromas superficiais, escutelares, dimidiados, orbiculares, radiados, 90—100 μ de diam., com área central esclarecida e margens marron-negras, fimbriadas, textura membranosa, ostíolo circular, 3.5—6.5 μ diam.; parede superior de 5—10 μ de espessura; himênio invertido; parede basal inobservada. Conidióforos filiformes, curtos, 1.5—2.5 × 1 μ. Picnidiosporos bacilares, contínuos, hialinos, 1—1.5 × 0.5—1 μ.

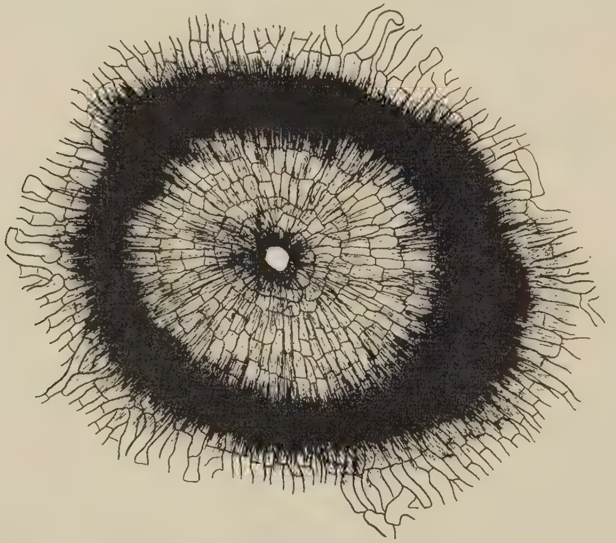


Fig. 30. *Peltasterella camaragibeana* Batista & Maia n. sp. Picnostroma.

Sôbre folhas vivas de *Ocotea* sp. associado a *Lembosia ocoteae* Bat. & Maia. Camaragibe, São Lourenço. Leg. Manuel R. Pereira 6.6.55. Tipo 2413, Instituto de Micologia, Universidade do Recife.

Mycelium amphigenum, superficiale, asterinoide, ex hyphis reticulatis, atro-brunneis, septatis, ex cellulis 12.5—20 × 3.5—5 μ, non setosis, compositum. Hyphopodia mucronata, alternata vel opposita, unicellularia, recta vel non, 12.5—16 × 2.5—4 μ. Pycnostromata superficialia, scutata, dimidiata, orbicularia, radiata, 90—100 μ diam., area centrali brunnescenti et marginibus atrobrunneis, fimfriatis; ostiolo centrali, 3.5—6.5 μ diam.; pariete superiore membranoso, 5—10 μ cr.; pariete basali non viso. Hymenium inversum. Conidiophoris filiformibus, 1.5—2.5 × 1 μ, hyalinis. Pycnidiosporae bacillares, continuae, hyalinae, 1—1.5 × 0.5—1 μ. — In foliis vivis *Ocoteae* sp. socia *Lembosiae ocoteae* nobis. Camaragibe, São Lourenço. Leg. Manoel R. Pereira, 6.6.55. Typus 2413, Instituto de Micologia, Universidade do Recife, Prov. Pernambuco, Brasil, Amer. Austr.

Peltasterella glochidiae Bat. & Maia n. sp.

Micélio epífilo, de hifas sinuosas, ramificações, marron-claras, septadas, com células de 24—27 × 2.5—3 μ; hifopodios laterais, alternos, raro opostos, globosos ou lobulares, unicelulares, 8—12 × 5—6.5 μ. Picnidios orbiculares, plano- escutelares, 73—95 μ de diam, 12—24 μ de alt, marron-claros, radiados com paredes de células poligonais, 4—9 × 4—10 μ, não setosos, ástomos; himenio invertido. Conidióforos muito curtos, até 3 μ de comprimento. Picnidiosporos bacilares a cilindráceos, hialinos, lisos, continuos, 2.5—3 × 1—2 μ.

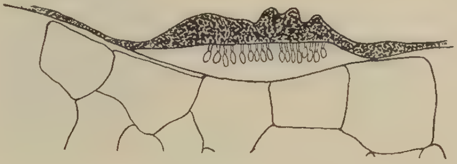


Fig. 31. *Peltasterella glochidiae* Batista & Maia n. sp.
Córte longitudinal de picnidio, observando-se o himenio invertido.

Sôbre folhas de *Glochidium ramosii* associado a *Asterina cassiae* Syd. var. *glochidiae* n. var. e *Meliola* sp. Bataan, Filipinas & Leg. P. W. Graff. XI, 1912. Tipo, 1308, F. Petrak, Mycotheca Generalis, no Magyar Nemzetti Museum — Hungria.

Mycelium epiphyllum, ex hyphis sinuosis, ramosis, brunnescentibus, septatis, ex cellulis 24—27 × 2.5—3 μ, compositum. Hyphopodia lateralia, alternata vel opposita, globosa vel lobata, unicellularia, 8—12 × 5—6.5 μ. Pycnostromata orbiculata, plano-scutata, 73—95 μ diam., 12—24 μ alt., brunnescentia; parietibus radiatis, glabris, astomis, ex cellulis 4—9 × 4—10 μ compositis; hymenium inversum. Conidiophoris usque 3 μ longis. Pycnidiosporae cylindraceae, continuae, hyalinae, 2.5—3 × 1—2 μ.

In foliis *Glochidii ramosii*, socia *Asterinae cassiae* Syd. var. glochidiae μ var. et *Meliolae* sp. Bataan, Philipp. Leg. P. W. Graff. XI, 1912. Typus, 1308, F. Petrak, Mycotheca Generalis, in Magyar Nemzetti Museum Hungr.

Peltasterella lembosicola Bat. & Maia n. sp.

Micélio epifilo, superficial, marron, de hifas sinuosas, septadas, com células de 17.5—25 × 2.5—4 μ, não setoso. Hifopodios laterais, nodulares ou subglobosos, unicelulares, irregularmente espalhados, 5—7.5 × 2.5—7.5 μ. Picnostromas superficiais, escutelares dimidiados, orbiculares, 32.5—50 μ de diam, 20—25 μ de alt. marron-negros, glabros, membranosos; parede superior radiada, 6—12 μ de espessura, com celulas de 3—4 × 2.5—3 μ; ostiolo de 1—2.5 μ de diam; parede inferior indistinta. Himenio invertido.

Conidioforos filiformes, 3—5.5 × 1 μ. Picnidiosporos bacilares ou cilindraceos, continuos, hialinos, 3—4 × 0.5—1 μ.

Sôbre folhas de *Didymopanax morototoni*, associado a *Lembosiellina recifensis* nobis. Dois Irmãos. Recife Leg. H. S. Maia, 12.9.56. Tipo, 5782, Instituto de Micologia, Universidade do Recife.

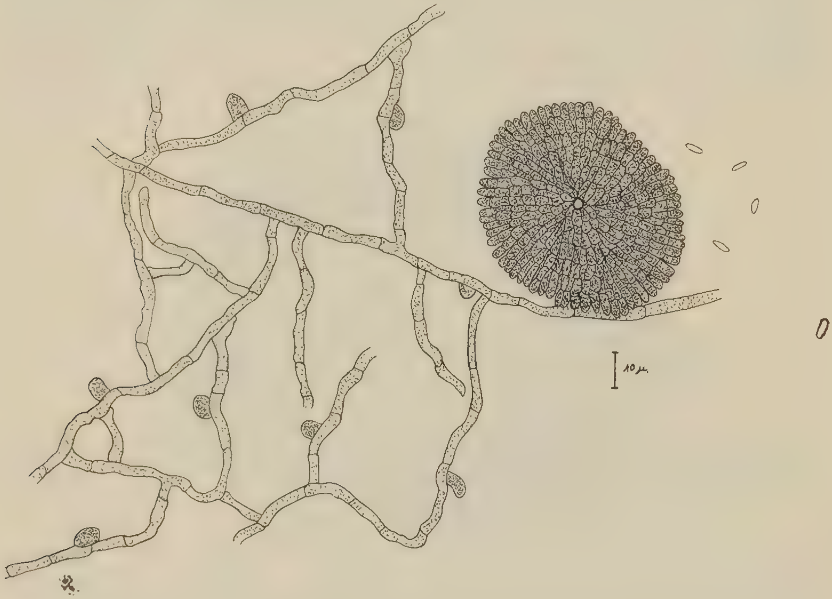


Fig. 32. *Peltasterella lembosicola* Batista & Maia n. sp.
Micélio hifopodiado, picnostroma e picnidiosporos.

Mycelium epiphyllum, superficiale, brunneum, ex hyphis sinuosis, septatis, ex cellulis 17.5—25 × 2.5—4 μ, non setosis, compositum. Hyphopodia lateralia, nodulata, vel globosa, ûnicellularia, irregulariter sparsa, 5—7.5 × 2.5—7.5 μ, efformata. Pycnostromata

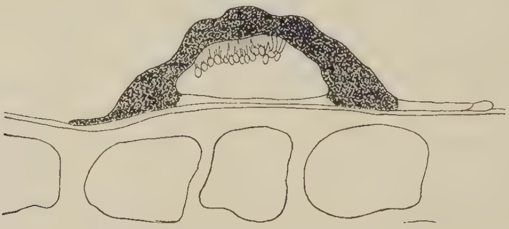


Fig. 33. *Peltasterella lembosicola* Bat. & Maia
Córte longitudinal do picnostroma.

superficialia, scutato-dimidiata, orbicularia, 32.5—50 μ diam., 20—25 μ alt., 6—12 μ cr., atro-brunnea, glabrata, membranosa, ostiolo centrali, 1—2.5 μ diam., pariete superiore radiato, ex cellulis 3—4 × 2.5—3 μ composito; pariete inferiore indistincto. Hymenium inversum. Conidiophoris filiformibus, 3—5.5 × 1 μ. Pycnidiosporae bacillares vel cylindraceae, continuae, hyalinae, 3—4 × 0.5—1 μ.

In foliis vivis *Didymopanacis morototoni* socia *Lembosiellinae recifencis*, nobis — Dois Irmaos — Recife — Leg. H. S. MAIA, 12.9.56. Typus, 5782, Instituto de Micologia, Universidade do Recife, Prov. Pernambuco, Brasil, Amer. Austr.

Associado tambem a *Asterina buddleifolii* BAT. & MAIA nº 5911, Instituto de Micologia, Universidade do Recife.

Peltasterella ocoteae BAT. & MAIA, n. sp.

Micélio hipófilo, superficial, asterinoide, de hifas sinuosas, crucialmente ramificadas, septadas, com células de 10—17 × 2—3 μ. Hifopódios alternos ou unilaterais, cilindráceos ou lageniformes, unicelulares, 6.5—10.5 × 2—3.5 μ. Picnostromas dispersos, superficiais, orbiculares, escutelar-dimidiados, 60—90 μ de diam., radiados, marron-claros, com ostíolo central de 2.5—5 μ de diam.; parede superior carbonácea, 5—6.5 μ de espessura, com células retangulares e poliédricas, 2.5—4 μ no maior diam., e margens fimbriadas; parede basal quase indistinta. Himênio invertido. Conidió-

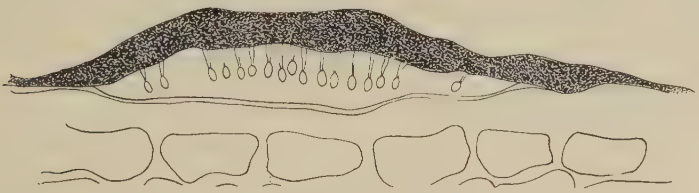


Fig. 34. *Peltasterella ocoteae* BAT. & MAIA

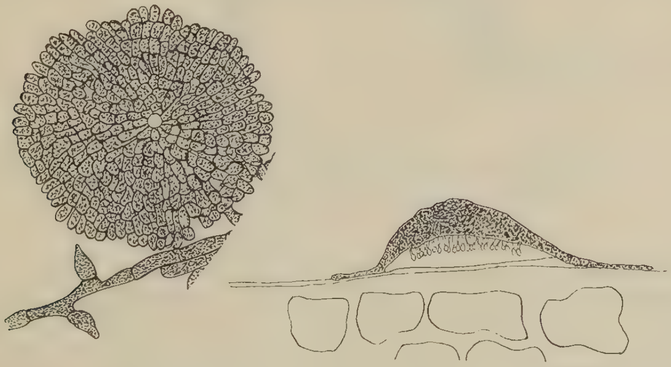


Fig. 35. *Peltasterella ocoteae* BAT. & MAIA
a) Picnidio e hifas do micélio com hifopódios lageniformes; b) córte longitudinal de picnidio exibindo himenio invertido. Orig.

foros filiformes, hialinos, 3—6 × 1—1.5 μ. Picnidiosporos cilindráceos, ou elipsoides, contínuos, hialinos, 3—5.5 × 2.5—3.5 μ.

Sôbre folhas vivas de *Ocotea* sp. associado a *Morenoella vitalii*, nobis. Dois Irmãos, Recife. Leg. OSVALDO SOARES DA SILVA, 1.11.55. Tipo 5016, Instituto de Micologia, Universidade do Recife.

Mycelium hypophyllum, superficiale, asterinoide, ex hyphis sinuosis, crucialiter ramosis, septatis, ex cellulis 10—17 × 2—3 μ, non setosis, compositum. Hyphopodia alternata vel unilateralia, cylindracea vel lageniformia, unicellularia, 6.5—10.5 × 2—3.5 μ. Pycnostromata sparsa, orbicularia, scutato-dimidiata, 60—90 μ diam., radiata, brunnescentia; ostiolo centrali; pariete superiore carbonaceo, 5—6.5 μ cr., ex cellulis rectangularibus vel polyedricis, 2.5—4 μ in long. diam. marginibus fimbriatis composito. Hymenium inversum. Pariete basali quasi indistincto. Conidiophoris filiformibus, hyalinis, 3—6 × 1—1.5 μ. Pycnidiosporae cylindraceae, vel ellipsoideae, continuae, hyalinae, 3—5.5 × 2.5—3.5 μ. — In foliis *Ocoteae* sp. socia *Morenoelli vitalii*, nobis. Dois Irmãos, Recife. Leg. OSVALDO SOARES DA SILVA, 1.11.55. Typus 5016, Instituto de Micologia, Universidade do Recife, Prov. Pernambuco, Brasil, Amer. Austr.

Peltasterella piriensis BAT. & MAIA n. sp.

Micélio superficial epifilo, marron-claro, não setoso, com hifas telemorficas, septadas, com celulas de 13—30 × 2.5—3 μ, ramificado alterna e unilateralmente em angulo de 45°. Hifopódios laterais, nodulares a capitados, unicelulares, alternos ou unilateralmente dispostos, 5.5—7 μ diam. Picnostromas marron-negros, circulares, isolados ou confluentes, 54—65 μ de diam, 16—27 μ alt, com paredes simples, 5—10 μ de espessura, compostas por células poligonais radialmente dispostas, 8—10 × 2.5—5.5 μ, ostiolo 2.5—3 μ diam. Parede inferior pouco nitida, Himenio invertido conidioforos filiformes, simples, continuos, hialinos, 3—5 × 0.5—1 μ. Picnidiosporos hialinos, bacilares a cilindraceos, continuos, 1.2—2.5 × 0.5—1 μ.

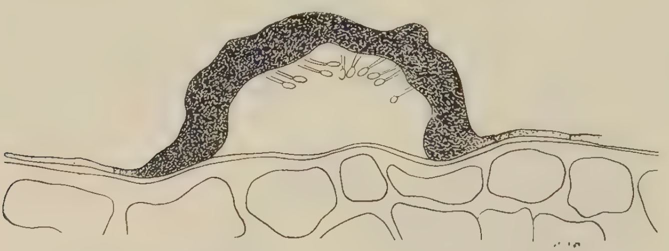


Fig. 36. *Peltasterella piriensis* BAT. & MAIA n. sp. Picnostroma observado con córte longitudinal.

Sôbre folhas de *Trichocladus ellipticus* Pirie Forest, Cape, coletado por E. M. DOIDGE, 18.7.927. Tipo nº 22427, Mycological Herbarium, Dep. Agr. Union of South Africa, associado a *Lembosia piriensis* e *Isipinga contorta* DOIDGE.

Obs.: DOIDGE (Bothalia IV, pag. 320, 1942) descreveu *Lembosia piriensis* indicando a presença de picnidios hemisfericos, de 60—100μ de diam e picnidiosporos bacilares, até 5 μ de extensão e 0.5 μ de diam. Evidentemente não verificou a presença de himênio invertido ou êsses picnidios representam uma forma distinta deste nosso. Peltasterella, cujas dimensões, alias, são bem menores.

Mycelium superficiale, epiphyllum, ex hyphis brunnescentibus, septatis, ramosis, ex cellulis cylindraceis, 13—30 × 2.5—3 μ, non setosis, compositum. Hyphopodia nodulata vel capitata, 1—2 cellularia, lateraliter efformata. Pycnostromata rotunda, libera vel confluentia, 54—65 μ diam., 16—27 μ alt., atro-brunnea; pariete superiore 5—10 μ cr., ex cellulis polygonalibus, 8—10 × 2.5—5.5 μ, radialiter dispositis composito; ostiolo pertuso, 2.5—3 μ diam. Pariete inferiore parvum nitidulo. Hymenium inversum. Conidiophoris simplicibus, continuis, hyalinis, 3—5 × 0.5—1 μ. Pycnidiosporae bacillares vel cylindraceae, continuae, hyalinae, 1.2—2.5 × 0.5—1 μ In foliis *Trichocladi elliptici* Pirie Forest, Cape. Leg. E. M. DOIDGE, 18.7.927. Typus, 22427, Myc. Herb. Dep. Agric. Union of South Africa, Pretória.

Peltasterella spissae BAT. & MAIA n. sp.

Micélio de *Parasterina spissa* (SYD.) BAT. & MAIA. Picnostromas superficiais, espalhados ou confluentes, circulares, dimidiados, 27—32.5 μ diam, 10—13 μ alt., marron, com parede superior de 3—5.5 μ, formada por células retangulares, radialmente dispostas, 8—10 × 5.5—8 μ diam.; dehiscência em fenda estelar e margens crenuladas. Himenio invertido; parede basal inobservada. Conidioforos quase obsoletos. Picnidiosporos hialinos, bacilares continuos, 1.2—2 × 1.2 μ.

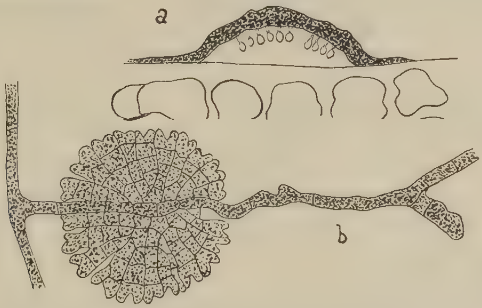


Fig. 37. *Peltasterella spissae* BATISTA & MAIA
a) picnostroma sob corte longitudinal; b) picnostroma sob vista frontal.

Sôbre folhas de *Jasminum* sp. Dodowa, Ghana, associado a *Parasterina spissa* (Syd.) Bat. & Maia e *Asterostomella walleniae* Petr. & Ciferri Leg. S. J. Hughes, 2.6.949. Tipo, 580, G.C.M. Herb.

Mycelium ut sub *Parasterina spissa* compositum. Pycnostromata sparsa vel confluentia, superficialia, plano-scutata, dimidiata, brunnea, 27—32.5 μ diam., 10—13 μ alt., parietibus 3—5.5 μ cr., ex cellulis rectangularis radialiter dispositis, 8—10 × 5.5—8 μ; stellata dehiscentia; marginibus crenulatis. Hymenium inversum; pariete basali non viso. Conidiophoris obsoletis. Pycnidiosporae bacillares, continuae, hyalinae, 1.2—2 × 1 μ.

In foliis *Jasmini* sp. socia *Parasterinae spissae* et *Asterostomella walleniae* Petr. & Cif. Dodowa, Ghana Leg. S. J. Hughes, 2.6.949. Typus, 580, G.C.M. Herb.

Asterostomella Speg. (Ann. Soc. Cien. Arg. 22: 198, 1886) emend. nobis.

Sin.: *Asteronia* Sacc. (Syll. Fung. 1: 47, 1882, como subgênero);
Hyphaster P. Henn. (Baum-Kun. Sambes Exp. 169, 1903).
Tipo: *A. paraguayensis* Speg. loc. cit.

Micélio superficial, não setoso, marron, reticulado ou radiante, de hifas septadas, com hifopodios. Picnostromas superficiais dimidiado-escutelares, radiados, de dehiscência estelar-irregular, marron. Himenio invertido. Conidioforos muito curtos ou obsoletos. Picnidiosporos ovoides, piriformes ou oblongos, continuos, marron, eventualmente com faixa hialina, transversal, gutulados ou não.

Obs.: Os resultados de nossas observações invalidam o conceito de E. M. Doidge de que seria possivel reconhecer especies de *Asterina* através da simples presença de *Asterostomella* spp. pois não existem de fato, quaisquer relações definidas entre êsses fungos capazes de ensejar tais diagnoses (Bothalia, IV: part II, 329, 1942).

ASTEROSTOMELLA — Espécies aceitas:

Chave baseada no tamanho dos picnostromas e dos picnidiosporos.

Picnostromas de 36—100 μ *de diam.*

	Picnostromas	Picnidiosporos
A. rhaphiostylidis Hansf. *in* Proc. Linn. Soc. 156 s.p. 118, 1994	80 μ diam.	20—25 × 18—21 μ
A. balanseana (K. & R.) Theiss *in* Rev. Mycol. pag. 76, 1890	36—80 μ diam.	13—18 × 7—9 μ
A. caricae P. Henn. *in* Hedw. pag. 391, 1904	40—90 μ diam.	9—14 × 6—9 μ
A. paraguayensis Speg. *in* Fung Guar. pag. 151	50—90 μ diam.	14—20 × 8—14 μ
A. capparidis Petrak *in* Ann. Myc. 27 : 396	50—100 μ diam.	12.5—24 × 15—18 μ

	Picnostromas	Picnidiosporos
A. walleniae PETRAK & CIFERRI *in* Ann. Myc. 30 : 265 . .	50—100 μ diam.	11—18 × 8—13 μ
A. indecora SYD. *in* Ann. Myc. 25 : 135	50—100 μ diam.	17—25 × 11—17 μ
A. erysiphoides (K. & C.) SACC. *in* Syll. Fung. 1 : 47, 1882.	60—100 μ diam.	18—20 × 10 μ
A. tremae SPEG. *in* Mycet. Agr. V. pag. 397, 1910	60—100 μ diam.	20—22 × 10—12 μ
A. veronicae (DESM.) ARN. *in* Ann. École. Nac. Agr. Montp.	60—100 μ diam.	11—16.5 × 8—10 μ
A. kutuensis (P. HENN.) BAT. & CIF. *in* Syll. Fung. XVIII : 685, 1906	100 μ diam.	20—30 × 15—18 μ

Picnostromas até 130 μ *de diam.*

	Picnostromas	Picnidiosporos
A. horridae BAT. & MAIA . .	85—110 μ diam.	19—24.5 × 13.5—19.5 μ
A. dispar SPEG. *in* Mycet. Arg. V. pag. 397, 1910	100—120 μ diam.	24—26 × 16—18 μ
A. strophanti P. HENN. *in* Engl. Jahrb. 38 : 125, 1905 . . .	70—120 μ diam.	16—22 × 10—14 μ
A. fusca BAT. *in* Bol. S.A.I.C. Pern XIII : 55, 1946 . . .	120—130 μ diam.	36 × 16 μ
A. tonduzi SYD. *in* Ann. Myc. 25 : 136, 1927	75—130 μ diam.	15—26 × 11—15 μ

Picnostromas até 150 μ *de diam.*

	Picnostromas	Picnidiosporos
A. orthosticha SYD. *in* Ann. Myc. 28 : 196	85—140 μ diam.	23—32 × 13—15 μ
A. acalyphae SYD. *in* Ann. Myc. 23 : 415	80—150 μ diam.	16—22 × 7.5—10 μ
A. cristata SPEG. *in* Mycet. Arg. V : 397, 1910	100—150 μ diam.	18—20 × 12—14 μ
A. cupaniae BAT. & CIF. . .	20—160 μ diam.	24—28 × 14—16 μ

Sem literatura

A. polystigma SYD.
in Philipp. Jour. Sci. XXI. nº 2, 145, 1922

A. aberiae PETCH
in Ann. Roy. Bot. Gard. Peradeniya VII part. 4 pag. 314, 1922

Nomen nudum

A. heteropteridis (P. HENN.) G. ARN.
in Ann. École Nat. Agr. Montp. XVI : 213, 1918 (nomen nudum)

Asterostomella acalyphae Syd.
in Ann. Myc. XXIII: 415, 1925.

Micélio de hifas onduladas, reticulado-ramificadas, marron-olivaceas, 3.5—5 μ de diam. Hifopodios 2-celulares, solitarios, 3—5 × 5—7.5 μ, lobados. Picnostromas de 80—150 μ de diam. Picnidiosporos oblongo-clavados, raro elipsoides, marron-olivaceos, com faixa subhialina, 16—22 × 7.5—10 μ.

Associado a *Asterina acalyphae* Syd. e *Asterina reticulata* Kalch. & Cke. (Bothalia, IV: part II, 299, 1942).

Asterostomella balanseana (K. & R.) Theiss.
in Rev. Myc. pag. 76, 1890.
Syll. Fung. IX: 390, 1891.
Sin.: *Asterostomella gregariella* Petr. & Cif.
in Ann. Myc. XXVIII: 406, 1930.

Micélio intricado-ramificado, com hifas de 6—7 μ de diam. Hifopodios alternos, verruciformes, a lobados, 9 × 6 μ. Picnostromas de 60—80 μ de diam. Picnidiosporos ovoides, com faixa hialina, marron-negros, continuos, 13—18 × 7—9 μ.

Sôbre Solanaceae, America.

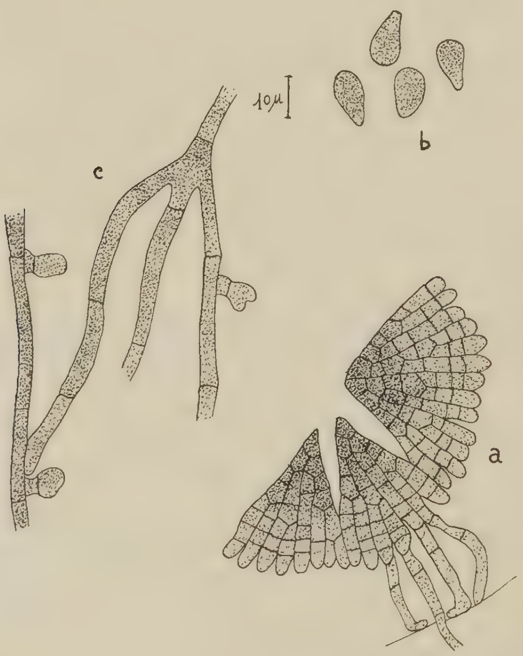


Fig. 38. *Asterostomella balanseana* (K. & R.) Theiss.
a) Sector do picnostroma; b) picnidiosporos; c) micélio hifopodiado.

Asterostomella capparidis PETRAK
in Ann. Myc. XXVII: 396, 1929.

Micélio reticulado; hifas de 3—4 μ de diam. Hifopodios bicelulares, cilindraceos, celula basal 4—5 × 1.5—2.5 μ e celula apical 5—9 × 4.5—6.5 μ. Picnostromas de 50—100 μ de diam. Picnidiosporos elipsoides, continuos, marron, 19.5—24 × 15—18 μ.

Associado a *Parasterina capparidis* (SYD. & BUTL.) BATISTA & MAIA.

Asterostomella caricae P. HENN.
in Hedw. pag. 391, 1904.

Plágulas e micélio superficial de *Asterina homaliicola*. Picnostromas dispersos, escutelares, orbiculares 40—90 μ diam, marron-negros, de dehiscencia em fenda estelar; parede superior formada por células retangulares, radialmente dispostas, 3—6 × 2.5—3 μ e bordos franjados, com células hifais de 7.5—19 × 2.5—3 μ, não

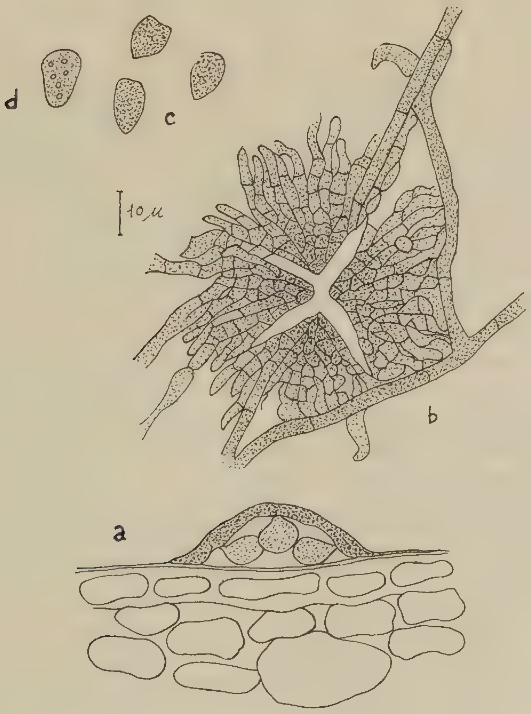


Fig. 39. *Asterostomella caricae* P. HENN.
a) picnostroma sob córte longitudinal; b) vista frontal do picnostroma e micélio; c) picnidiosporos; d) picnidiosporo com poros germinativos.

hifopodiadas; parede inferior subhialina, quase indistinta. Conidioforos ausentes. Himenio invertido. Picnidiosporos marron-claros, continuos, oblongos, ovais a piriformes e por vezes irregulares, angulosos com numerosos poros germinativos, 9—14 × 6—9 μ diam.

Sôbre folhas de *Homalium dolichophyllum* AGONA, Tarkwa, Ghana Leg. S. J. HUGHES, 12.5.949. Espec. nº, 879, em Ghana Myc. Herb. Lectotipo.

Obs.: HUGHES (Fungi from Ghana 1952) não designou êsse fungo, tendo-o descrito embora, incompletamente, como fórma imperfeita. Está associado tambem a *Asterina xumenensis* DOIDGE e *A. radiofissilis* DOIDGE (Bothalia, IV: part II, 295—309, 1942).

Asterostomella cristata SPEG.
in Mycet. Argent. V: 397, 1910.

Micélio fibriloso, fuligineo, com hifas de 4—6 μ de diam. Hifopodios orbiculares, 2—6 lobados, 10—15 × 7—15 μ. Picnostromas de 100—150 μ de diam. Picnidiosporos obovoides, continuos, 1-gutulados, fuligineos, 18—20 × 12—14 μ.

Sôbre *Helietta cuspidata*, America.

Asterostomella cupaniae BAT. & CIFERRI n. sp.

Micélio asterinoide. Hifopodios alternos, unicelulares, marron, 8—12 × 6—7.5 μ. Picnostromas orbiculares, plano-convexos, radiados, marron-negros, 20—160 μ de diam. Himenio invertido; parede basal inconspicua. Picnidiosporos ovoides ou piriformes, marron-negros, com faixa hialina transversal, continuos, 24—28 × 14—16 μ.

Sôbre fôlhas de *Cupania revoluta* RADLLS associado a *Parasterina cupaniae* BAT. 17/7/949. Tipo, nº 11317, Instituto de Micologia Universidade do Recife.

Mycelium asterinoideum. Hyphopodia alterna, continua, irregulariformia, brunnea, 8—12 × 6—7,5 μ. Pycnostromata orbicularia, plano-convexa, radiata, atro-brunnea, 20—160 μ diam.; hymenium inversum; pariete basali inconspicuo. Pycnidiosporae ovoideae vel pyriformes, atro-brunneae, cum fascia transversa hyalina, continua, 24—28 × 14—16 μ.

In foliis *Cupaniae revolutae*, socia *Parasterinae cupaniae*, 17/7/949. Typus, 11371, Instituto de Micologia, Univ. do Recife.

Asterostomella dispar SPEGAZZINI
in Mycet. Arg. V: 397, 1910.

Micélio tenue, de hifas retas, 5—6 μ de diam. Hifopodios alternos, cilindraceos. Picnostromas de 100—120 μ de diam. Picnidiosporos oblongos, continuos, marron, 24—26 × 16—18 μ.

Sôbre *Styrax leprosa*, Argentina. Associado a *Asterina disparis* SPEG.

Asterostomella erysiphoides (KALCH. & CKE.) SACC.
in Syll. Fung. I: 47, 1882.
Sub. *Asterina erysiphoides* KALCH. & CKE.

Micélio tenue, ramificado, hifopodiado, fusco. Picnostromas de 60—100 μ de diam., Picnidiosporos elipticos, continuos, marron-negros, 18—20 × 10.

Sôbre *Jasminum tortuosum*, Africa meridional.

Associado tambem a *Asterina vanderbylii* WERD (Bothalia, IV: part II, 308, 1942) e *Asterina crotoniensis* (DOIDGE) RYAN (Bothalia, IV: part. II, 293, 1942).

Asterostomella fusca BATISTA
in Bol. Sec. Agr. Pern. XIII: 55, 1946.

Micélio reticulado, marron, de hifas septadas, 4—5 μ de diam. Hifopodios subglobosos, continuos, 6—9 μ de diam, alternos ou unilaterais. Picnostromas escutelares, radiados, 120—130 μ de diam; himenio invertido. Conidioforos quase obsoletos. Picnidiosporos sesseis, ovoides ou oblongos, continuos, 36 × 16 μ.

Sôbre *Rollinia silvatica*, Brasil.

Asterostomella horrida BAT. & MAIA n. sp.

Plágulas anfigenas, crustaceas, marron-negras, arredondadas, 4—9 mm de diam, ou confluentes, até 30 mms de extensão. Micélio reticulado, de hifas subretas, ramificadas em angulos de 45°, unilateral e alternadamente, com células de 16.5—19 × 3—4 μ. Hifopodios, capitados, bicelulares, globosos ou cilindraceos, retos ou curvos, opostos ou unilaterais, 7.5—9 × 5—6.5 μ. Picnostromas circulares, 85—110 μ de diam, dispersos, escutelares, carbonaceos, de dehiscencia estelar e bordos franjados; parede superior radiada, com células de 4—5 × 3—4 μ; parede basal indistinta. Picnidiosporos ovoides, amarelos a principio depois marron, continuos, lisos, 19—24.5 × 13.5—19.5 μ.

Sôbre *Capparis horrida* — Los Baños, Luzón, Philippines, Março 1914. Tipo, nº 506, Magyar Nemzetti Museum, Budapest.

Obs.: Esse especimen que obtivemos para estudo mediante a generosidade do Dr. BOHUS GÁBOR, da Hungria, embora det. por SYDOW como *Asterina capparidis* SYD. & BUTL. somente possue picnostromas. A diagnose original de SYDOW & BUTLER (Ann. Mycol. IX: 390, 1911) não menciona picnostromas e assim definimos êsse *Asterostomella*, admitindo-o como estado imperfeito do *Parasterina capparidis* (SYD. & BUTL.) BATISTA & MAIA.

Plagulae amphigenae, crustaceae, atro-brunneae, rotundatae, 4—9 mm diam. vel confluentes usque 30 μ long. Mycelium reticulatum, ex hyphis subrectis, ramosis, ex cellulis 16.5—19 × 3—4 μ, compositum. Hyphopodia capitata, bicellularia, globosa vel cylindracea, recta vel incurvata, unilateralia vel opposita, 7.5—9 × 5—6.5

μ. Pycnostromata circularia 85—110 μ diam., sparsa, scutata, marginibus fimbriatis, stellata dehiscentia; pariete superiore radiato, ex cellulis 4—5 × 3—4 μ efformato. Pycnidiosporae ovoideae, primo flavidae dein brunneae, continuae, leves, 19—24.5 × 13.5—19.5 μ.

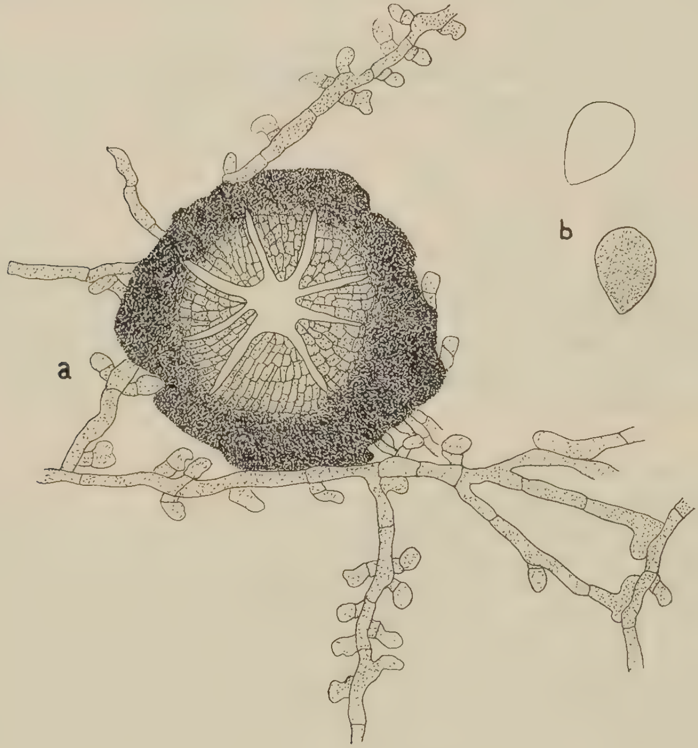


Fig. 40. *Asterostomella horrida* BAT. & MAIA n. sp.
a) Picnostroma e micélio hifopodiado; b) picnidiosporos. Orig.

In foliis *Capparis horridae* — Los Baños, Luzón, Philippines, 3/914. Typus, 506, Magyar Nemzetti Museum, Budapest. Status imperf. *Asterina capparidis* SYD. & BUTL.

Asterostomella indecora SYD.
in Ann. Myc. 25: 135, 1927.

Micélio reticulado, de hifas irregularmente ramificadas, 3—5 μ de diam. Hifopodios continuos, pluri-lobados, 7.5—11 × 8—12 μ. Picnostromas 50—100 μ de diam. Picnidiosporos, ovoides, piriformes, raramente subglobosos ou angulares, marron-negros, 17—25 × 11—17 μ.

Sôbre *Malpighia glabra*, Costa Rica.

Associado a *Parasterina indecora* (SYD.) BATISTA. (loc. cit.), *Asterina delicata* DOIDGE (Bothalia IV: part II, 292, 1942), *Asterina*

capparidicola DOIDGE, *Asterina elegans* DOIDGE e *Asterina scolopiae* DOIDGE (Bothalia IV: part II, 311, 299, 1942).

Asterostomella kutuensis (P. HENN.) n. comb.
Sin.: *Hyphaster kutuensis* P. HENN.
in Ergebn. der Kunene Sambesi Expedit. pag. 169, 1902; Syll. Fung. XVIII: 685, 1906.

Micélio ramificado, reticulado, de hifas septadas, 3—5 μ de diam, marron. Hifopodios lobados. Picnostromas orbiculares, negros, 100 μ de diam. Himenio invertido. Picnidiosporos ovoides, continuos marron-negros, com faixa hialina, 20—30 × 15—18 μ.
Sôbre *Combretum*, Africa.

Asterostomella orthosticha SYD.
in Ann. Myc. XXVIII: 196, 1930.

Micélio de hifas irregularmente ramificadas, 4—6.5 μ de diam. Hifopodios continuos, solitarios, disciformes ou cilindraceos, 7.5—11 × 6—7.5 μ. Picnostromas de 85—140 μ de diam. Picnidiosporos ovoides ou piriformes, continuos, 2-gutulados, marron-negros, 23—32 × 13—15 μ.
Sôbre *Doliocarpus*, Venezuela.
Associado a *Asterina acalyphae* SYD. (loc. cit.) e *Asterina hendersoni* DOIDGE, *in* Bothalia, IV: part II, 290, 1942.

Asterostomella paraguayensis SPEG.
in Fung. Guar. p. 151.
Sin.: *Asterostomella isothea* SYD.
in Ann. Myc. XXV: 135, 1927.
Asterostomella dorsteniae SYD.
in Ann. Myc. XXV: 134, 1927.
Asterostomella banisteriae SYD.
in Ann. Myc. XXVII: 78, 1929.
Asterostomella africana SYD.
in Engl. Bot. Jarb. 45: 264, 1910.
Asterostomella concinna SYD.
in Ann. Myc. XXVIII: 196, 1930.

Micélio hifopodiado. Picnostromas de 50—90 μ diam. e picnidiosporos de 14—20 × 8—14 μ.
Sôbre folhas de *Cestrum*, *Triumfetta semitriloba* e Rutaceae.
Obs.: Está associado tambem a *Asterina pavoniae* WERD, *Asterina africana* (VAN DER BYL) DOIDGE, *Asterina diplocarpa* CKE., *Asterina polythyria* DOIDGE, *Asterina grewiae* CKE. (Bothalia, IV: part II, pag. 295, 310, 293, 396, 303, 1942).

Asterostomella rhaphiostylidis HANSF.
in Proc. Linn. Soc. Lond. 156 ses. part. II, pag. 118, 1943—4.

Anfigeno. Micélio de hifas flexuosas ou subretas, 3.5—4.5 μ de diam., frouxamente reticulado. Hifopodios alternos, unilaterais ou opostos, 2-celulares, 11—26 × 6—8 μ. Picnostromas de 80 μ de diam. Picnidiosporos subglobosos, marron-negros, continuos, 20—25 × 18—21 μ.

Sôbre *Rhaphiostylis beniensis*, Uganda.

Tambem associado a *Asterina excoecariae* DOIDGE (Bothalia, IV: part II, 307, 1942).

Asterostomella strophanti P. HENN.
in Engl. Jahrb. 38: 125, 1905.
Sin.: *Asterostomella visci* DOIDGE
in Bothalia IV: part II, 330, 1942.
Asterostomella eugeniicola DOIDGE
in Bothalia IV: part II, 338, 1942.

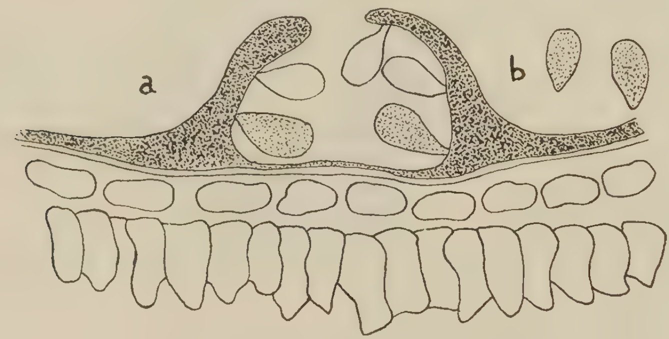


Fig. 41. *Asterostomella strophanti* P. HENN.
a) Córte longitudinal; b) picnidiosporos

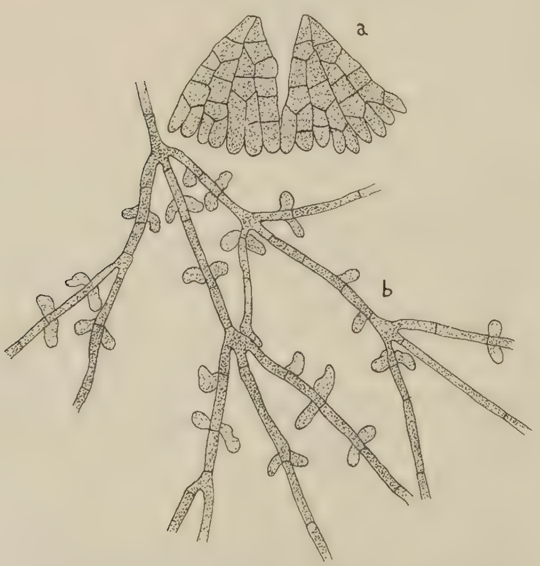


Fig. 42. *Asterostomella strophanti* P. HENN.
a) Sector do picnostroma; b) micélio hifopodiado.

Micélio hifopodiado. Picnostromas de 50—120 μ de diam. Picnidiosporos piriformes, continuos, marron, 15—21 × 10—12.5 μ.

Sôbre folhas de *Viscum obscurum* e *Eugenia natalitia*, Africa.

Tambem associado a *Asterina loranthicola* SYD. (Bothalia, IV: part. II, 302, 1942).

Igualmente assinalado sôbre *Flacourtia ramontchii* — Entebbe — Nº 3266, Herb. Mycologist, Dep. Agr. Uganda. Leg. C. G. HANSFORD XI, 1943. Em associação com *Parasterina greviae* CKE. var. *granulosa* HANSF., *Eriomycopsis trichiliae* HANSF. e *Phaeodimeriella parvula* (CKE.) HANSF.

Asterostomella tonduzi SYD.
in Ann. Myc. XXV: 136, 1927.
Sin.: *Asterostomella meliosmaticola* PETR. & CIF.
in Ann. Myc. XXX: 264, 1932.
Asterostomella forsteroniae (P. HENN.) G. ARN.
in Ann. Écol. Nat. Agric. Montp. XVI: 215, 1918.

Micélio de hifas reticulado-ramificadas, 3—5 μ de diam. Hifopodios bicelulares, 12—15 × 4—5.5 μ. Picnostromas de 75—130 μ de diam. Picnidiosporos oblongo-ovoides, marron-negros, continuos, 1—2 gutulados, 15—26 × 11—15 μ.

Sôbre *Xylosma velutina* — Costa Rica.

Associado a *Parasterina tonduzi* (SYD) RYAN e *Asterina combreti* SYD. (Bothalia, IV: part. II, 301, 1942).

Asterostomella tremae SPEG.
in Mycet. Arg. V. pag. 397, 1910.
Sin.: *Asterostomella ornata* CIF.
in Sydowia, VIII: 248, 1954.
Asterostomella minuta ARN.
in Ann. École Nat. Agr. Montp. XVI: 215, 1918.

Micélio hifopodiado. Picnostromas de 60—100 μ de diam; picnidiosporos de 20—22 × 10—12 μ, continuos, marron.

Sôbre folhas de *Trema micranthum* e *Fevillea cordifolia*, America.

Asterostomella veronicae (DESM.) ARN.
in École Nat. Agr. Montp. 16: 174, 1918.
Sin.: *Asteroma veronicae* DESM.
in Pl. Crypt. ed. I, fasc. XVI, nº 788, 1836.
Asteroma veronicarum RABH.
in Herb. Myc. ed. II, nº 739.

Plágulas anfígenas, efusas, marron-negras e membranosas. Micélio anfígeno, superficial, marron-oliváceo, não setoso telemórfico, septado, não constricto, com ramificações opostas, alternas e unilaterais, regulares, em ângulos de 45—90°; células hifais de 13.5—21.5 × 5.5—8 μ. Hifopódios alternos e unilaterais, capitados, simples ou multilobados, encurvados, unicelulares, 6.5—11 × 5.5—10.5 μ. Picnostromas anfígenos, superficiais, circulares a subcirculares, marron-oliváceo, isolados, membranosos, de dehiscência estelar, 61.5—100 μ de diam., 36.5—48.5 μ de alt., sub-parenquimáticos, de parede superior radiada, 8—10 μ de espessura, formada por células retangulares, poligonais, 8—11 × 5.5—8 μ; himênio invertido; membrana basal inconspícua. Conidióforos cilindráceos, hialinos, lisos, muito curtos, 1.5—2.5 μ de extensão. Picnidiosporos piriformes, não septados, lisos, marron-claros, apresentando uma faixa transversal sub-hialina, 11—16.5 × 8—10 μ.

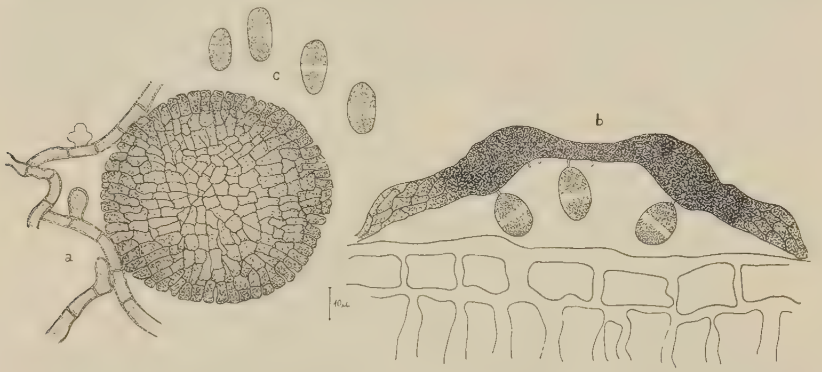


Fig. 43. *Asterostomella veronicae* (DESM.) ARN.
a) Picnostroma e micélio hifopodiado; b) picnostroma sob córte longitudinal; c) picnidiosporos. Orig.

Sôbre folhas de *Veronica derwentiae* LITTLEY, associado a *Parasterina veronicae* (LIB.) ARN. e *Ramalia veronicae* nobis n. sp. Clyde Mountain, N. South Wales. Leg. L. FRASER, Jan. 1937. Espec. nº 211, Dep. Bot. University of Sydney, New South Wales, Det. A. CHAVES BATISTA, em 28.8.957.

Asterostomella walleniae PETRAK & CIFERRI
in Ann. Myc. XXX: 265, 1932.
Sin.: *Asterostomella peraffinis* SPEG.
in F. Puigg. nº 434, 1889.
Asterostomella lismorensis SYD.
in Ann. Myc. XXXV: 49, 1937.
Asterostomella hamatula CIF.
in Ann. Mycol. XXXVI: 230, 1938.

Micélio hifopodiado. Picnostromas superficiais, marron-negros, circulares, dimidiados, 36.5—80 μ diam isolados ou confluentes; parede superior de células retangulares, radialmente dispostas 3—5.5 × 2.5—3 μ; bordos franjados com células de 6.5—11.5 × 2.5—3 μ e dehiscência em fenda estelar; himenio invertido, parede basal inobservada. Picnidiosporos sesseis, marron-escuros, lisos, unicelulares, piriformes, 11—18 × 8—13 μ.

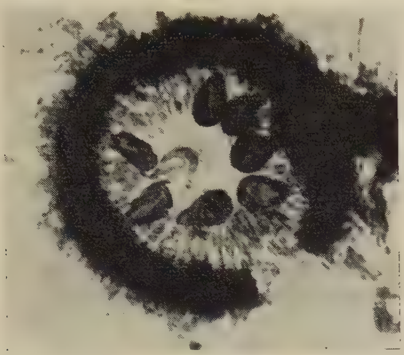


Fig. 44. *Asterostomella walleniae* Petr. & Cif.
Picnostroma sob córte transversal. Orig.

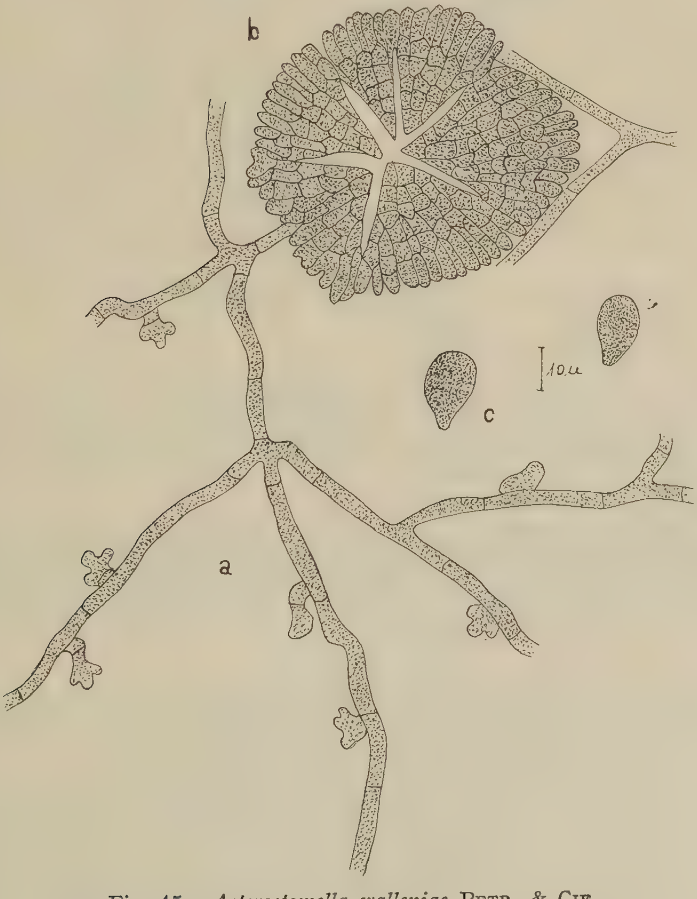


Fig. 45. *Asterostomella walleniae* Petr. & Cif.
a) Micélio hifopodiado; b) picnostroma; c) picnidiosporos. Orig.

Sôbre folhas de *Justicia flava*, associado a *Parasterina concinna* (Syd) Bat. & Maia. Bumsu, Ghana, S. J. Hughes, 7.6.947. Esp. nº 20, G.C.M. Herb.

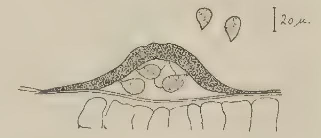


Fig. 46. *Asterostomella walleniae* Petr. & Cif.
Picnostroma sob córte longitudinal.

Sôbre folhas de *Pleioceras barteri* associado a *Asterina pleioceratis* Hughes — Esiama, Ghana Leg. S. J. Hughes, 14.5.949. Espec. nº 924, no Ghana Mycol. Herb.

Tambem associado a *Asterina clausenicola* Doidge (Bothalia IV: part. II: 303, 1942).

Está associado tambem a *Asterina rinorae* Doidge e *Asterina tertia* Rac. var. *africana* Doidge (Bothalia, IV: part II, pag. 312 e 305, 1942).

Obs.: Elegemos *A. walleniœ* Petrak & Ciferri por sua melhor representaçâo das caracteristicas dêsse fungo, como binomio válido, em face de os especimens que suportam as designaçôes ora sinonimas ja se encontrarem em condiçôes desfavoraveis para o estudo.

Asterostomopsis Bat. & Ciferri & Maia n. gen.

Tipo: *A. ghanaensis* nobis.

Micélio superficial, marron, hifopodiado, não setoso. Picnostromas escutelares, radiados, marron, de dehiscencia estelar; himenio invertido; hipostroma simples. Picnidiosporos continuos, marron com faixa hialina ou não.

Typus: *A. ghanaensis* nobis.

Mycelium superficiale, brunneum, hyphopodiatum, non setosum. Pycnostromata scutata, radiata, stellata dehiscentia; hymenium inversum; hypostromata simplicia. Pycnidiosporae continuae, brunneae, cum zonis hyalinis vel non.

Asterostomopsis ghanaensis Bat. & Ciferri & Maia n. sp.

(Em homenagem ao país de origem, que se tornou independente agora, no Continente Africano, Abril 1957).

Plágulas anfigenas, marron-escuras, crustaceas, diminutas, isoladas ou não, 1—2 mm de diam. Micélio superficial, não setoso, com hifas telemorficas, septadas, hifopodiadas, ramificadas opostamente em angulos de 45°, com células de 11—19 × 5.5—6.5 µ. Hifopodios laterais, nodulares a subcilindraceos, 1—3 lobados, unicelulares 5.5—11 × 5.5—8 µ. Hipostroma cilindrico, formado por hifas marron-claras, septadas, dispostas em cunha, penetrando até o mesófilo e partindo da porção basal do picnostroma, 12—14 × 10—13 μ. Picnostromas escutelares, orbiculares, 73—170 μ de diam, isolados ou confluentes, marron, astomos, de dehiscencia em fenda

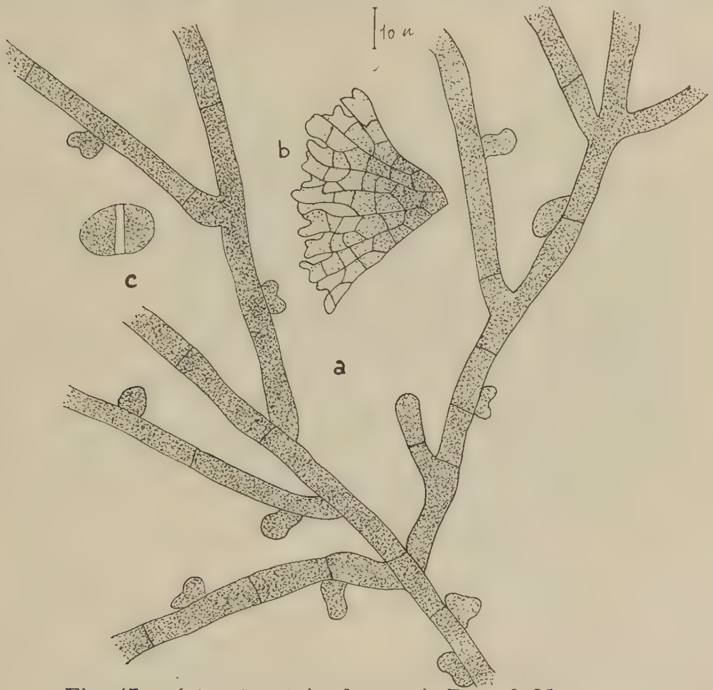


Fig. 47. *Asterostomopsis ghanaensis* Bat. & Maia n. sp.
a) Micelio hifopodiado; b) sector do picnostroma; c) picnidiosporo.

estelar; parede superior de células retangulares radialmente dispostas de 6.5—8 × 3—5.5 μ, de bordos não franjados. Himenio invertido; parede basal tenue, marron, conectada com o hipostroma. Conidioforos obsoletos. Picnidiosporos marron-claros continuos, piriformes a sub-globosos, lisos, 13.5—19 × 6.5—8 μ, com uma faixa mediana hialina.

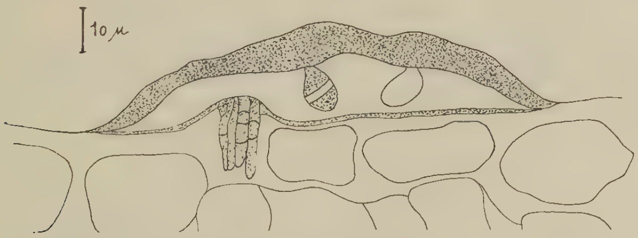


Fig. 48. *Asterostomopsis ghanaensis* Bat. & Maia n. sp.
Picnostroma sob córte longitudinal, observando-se o hipostroma.

Sôbre folhas de *Lawsonia inermis* Tafo, Ghana. Leg. NOEL ROBERTSON, 23.2.947. Tipo, nº 718, Ghana Myc. Herb. e 42.198 no Commonwealth Mycol. Institute, Inglaterra.

Obs.: O especimen em causa achava-se sob a etiqueta de *Asterina lawsoniae* HENN. & NYM. A histologia, entretanto, revelou-nos que não se trata dessa especie, pois os picnostromas possuem hipostroma e assim a erigimos como tipo de um novo gênero.

Plagulae amphigenae, atro-brunneae, crustaceae, minutae, 1—2 mm diam. Mycelium superficiale, ex hyphis opposite ramosis in angulis 45°, septatis, brunneis, ex cellulis 11—19 × 5.5—6.5 μ, compositum non setosum. Hyphopodia lateralia, nodulata, vel subcylindrica, unicellularia, 1—3-lobata, 5.5—11 × 5.5—8 μ. Pycnostromata scutata, orbiculata, brunnea, 73—170 μ diam., isolata vel confluentia, stellatim dehiscentia; pariete superiore radiato, ex cellulis rectangularibus, 6.5—8 × 3—5.5 μ, marginibus non fimbriatis composito; hymenium inversum; pariete basali tenui, brunneo, affixo hypostromatis cylindricis, usque mesophyllo penetranti, brunneis, 12—14 × 10—13 μ, et hyphis septatis, parallelis compositis. Conidiophoris obsoletis. Pycnidiosporae continuae, oblongae vel pyriformes, leves, brunneae, in zona mediana hyalinae, 13.5—19 × 6.5—8 μ. In foliis *Lawsoniae inermis*, Tafo, Ghana. Leg. NOEL ROBERTSON, 23.2.947. Typus, 718, Ghana Myc. Herb. et 42198, Comm. Myc. Inst. Lond.

Rota BAT. & CIFERRI n. gen.

Tipo: *R. pulchra* BAT. & CIFERRI & NASC.

Micelio asterinoide, septado, pouco ramificado, hifopodiado, marron, não setoso. Picnostromas dispersos ou gregarios, superficiais, radiados, escutelares, circulares, de dehiscencia irregular, marron; membrana basal tenue, hialina; hipostroma multiplo, marron. Himenio invertido. Picnidiosporos sesseis, cilindraceo-fusoides, 1-septados, hialinos.

Typus *R. pulchra* BAT., CIFERRI & NASC.

Mycelium asterinoide, septatum, parvum ramosum, hyphopodiatum, brunneum, non setosum. Pycnostromata sparsa vel gregaria, superficialia, radiata, scutata, rotundata, irregulariter dehiscentia, brunnea; membrana basali tenui hyalina, hypostromatis pluribus affixa, brunneis. Hymenium inversum. Pycnidiosporae sessiles, cylindraceo-fusoideae, 1 septatae, hyalinae.

Rota pulchra BAT., CIFERRI & NASC. n. sp.

Plágulas hipofilas, arredondadas, livres ou confluentes, marron-negras, 1—4 mms, de diam. Micelio asterinoide, de hifas marron, septadas, não constrictas, ramificadas em angulos de 45°, tendo celulas de 12—50 × 3.5—6 μ, não setoso. Hifopodios laterais, unicelulares, direito ou recurvado, escassos, 5—7 × 3.5—5 μ, produzindo haustorios subcuticulares a epidermais. Picnostromas dispersos ou gregarios, plano-escutelares, orbiculares, 120—195 μ de diam, radiados, de margens ondeadas, marron; parede superior formada por células retangulares, 3.5—7 × 1.5—5 μ; membrana

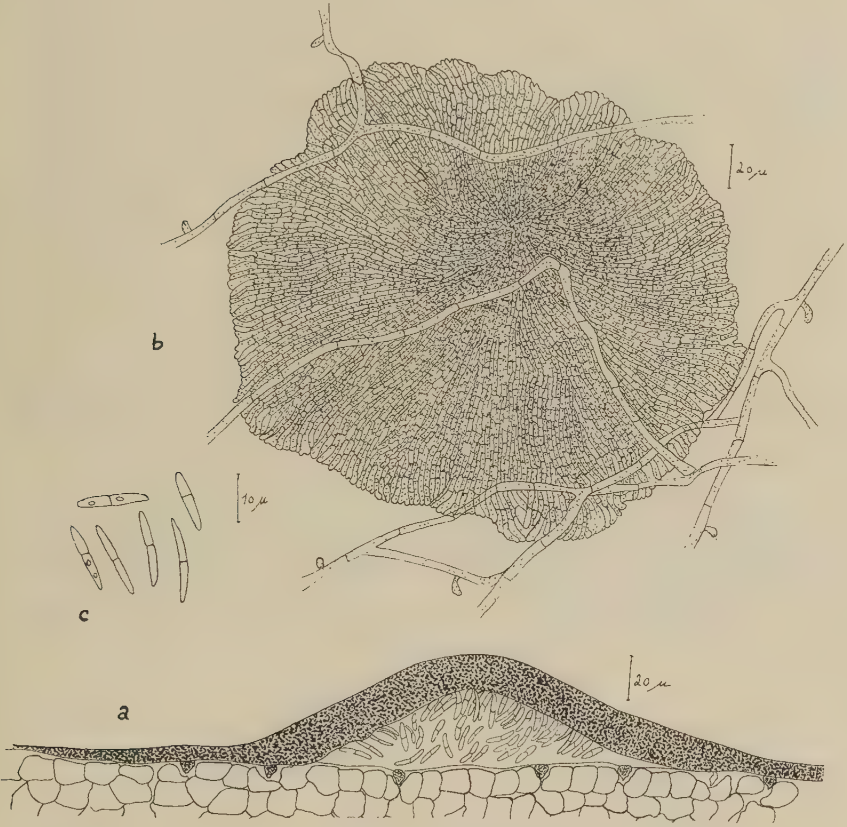


Fig. 49. *Rota pulchra* Bat. & Nasc. n. sp.
a) Picnostroma, sob córte longitudinal; b) picnostroma, sob vista frontal e micelio; c) picnidiosporos.

basal tenue, hialina 2—4 μ de diâmetro; hipostroma multiplo, subcuticular, conoide, 4.5—8.5 × 6—8.5 μ, marron, continuo. Himenio invertido. Picnidiosporos sesseis, cilindraceo-fusoides, 1-septados, não constrictos, gutulados, hialinos, 12—14 × 2—2.5 μ.

Sôbre folhas de hospedeiro desconhecido. Rio Ouro Preto, Mato Grosso, Brasil. Leg. J. R. Weir, 17/9/1923. Tipo, nº 11043, Instituto de Micologia, Universidade do Recife. Ex Fungi of the Amazon River Drainage, cedido pelo Dr. J. A. Stevenson, The National Fungus Collections, U.S.A.

Plagulae hypophyllae, rotundatae, isolatae vel confluentes, atro-brunneae, 1—4 mm. diam. Mycelium asterinoide, ex hyphis brunneis, septatis, non constrictis, ramosis in angulis 45°, ex cellulis 12—50 × 3.5—6 μ, non setosis, compositum. Hyphopodia lateralia, unicellularia, rara, recta vel incurvata, 5—7 × 3.5—5 μ. Pycnostromata sparsa vel gregaria, plano-scutata, orbiculata, 120—195 μ diam., radiata, marginibus ondulatis, brunnea; membrana basali tenui, hyalina, 2—4 μ diam. Hypostromata pluria, subcuticularia, conoidea, contínua, brunnea, 4.5—8.5 × 6—8.5 μ. Hymenium inversum. Pycnidiosporae sessiles, cylindraceo-fusoideae, 1-septatae, non constrictae, gutulatae, hyalinae, 12—14 × 2—2.5 μ.

In foliis plantae ignotae. Rio Ouro Preto, Mato Grosso, Brasil. Leg. JAMES R. WEIR, 17/9/923. Typus, n⁰ 11043, Instituto de Micologia, Universidade do Recife. Prov. Peru. Brasil, Amer. Austr. Ex Fungi of the Amazon River Drainage, The National Fungus Collections.

Manginulaceae n. fam. BAT. & CIFERRI

Picnostromas marron-negros, subcuticulares, destituidos de micélio livre, escutelares, de himenio invertido, radiados, ostiolados ou não, sem hipostroma, com esporos de várias fórmas, hialinos ou corados. Tipo **Manginula** ARN.

Mycelium liberum nullum; pycnostromata atro-brunnea, subcuticularia, non hypostromata, scutata, radiata, hymenio inverso, ostiolata vel non. Pycnidiosporae variae. Typus **Manginula** ARN.

Nessa familia agrupam-se algumas fórmas imperfeitas relacionadas com os Stigmateaceae.

CHAVE PARA OS GÊNEROS

HYALOAMEROSPOROS

A) Micélio livre ausente.

B) Picnostromas radiados; astomos; conidioforos paliçadiços; picnidiospores bacilares .... **Thyriostroma** DIED; (Ann. Myc. XI: 176, 1913)

BB) Picnostromas pseudoparenquimáticos; pseudo-ostiolados; conidioforos ausentes; picnidiosporos bacilares .... **Parastigmatellina** BAT. & COSTA

FEOAMEROSPOROS

Micélio livre ausente; estigmopodios subcuticulares; picnostromas radiados; astomos; picnidiosporos ovoides, com faixa hialina transversal ou não .... **Manginula** ARN. (Ann. Agr. Montp. XVI: 218, 1918)

HIALODIDIMOSPOROS

A) Micélio livre ausente; picnostromas radiados; dehiscencia estelar; conidioforos ausentes; picnidiosporos cilindricos fusoides .... **Stellothyriella** BAT. & CIF.

Parastigmatellina BAT. & COSTA n. gen.
Tipo: *P. asiatica* nobis.

Micelio livre ausente. Picnostromas, subcuticulares, escutelares, dimidiados, marron-negros, glabros, de textura compacta; hipostroma ausente; Himenio invertido; picnidiosporos sesseis, bacilares, continuos, hialinos, formados em substancia mucosa.

Typus: *P. asiatica*, nobis.
Mycelium nullum. Hypostromata nulla. Pycnostromata subcuticularia, scutata, dimidiata, atro-brunnea, glabrata, pseudoparenchymatica. Hymenium inversum. Pycnidiosporae sessiles, bacillares, continuae, hyalinae, in substancia mucosa evolutae.

Obs.: Este gênero difere de **Hansfordiopeltis** BAT. & COSTA pelos seus picnostromas subcuticulares, pseudo-parenquimaticos.

Parastigmatellina asiatica BAT. & COSTA n. sp.

Micélio ausente. Picnostromas epifilos, subcuticulares, dispersos escutelar-conoides, dimidiados, 130—185 μ de diam, 120—155 μ de alt, pseudo-ostiolados, marron-negros, opacos, pseudoparenquimáticos, parede superior de textura compacta, 36—48 μ de espessura, glabros; himenio invertido; parede basal indistinta; hipostroma ausente. Picnidiosporos sesseis, bacilares, continuos, lisos, hialinos, 3.5—5 × 1 μ, reunidos em substancia mucosa.

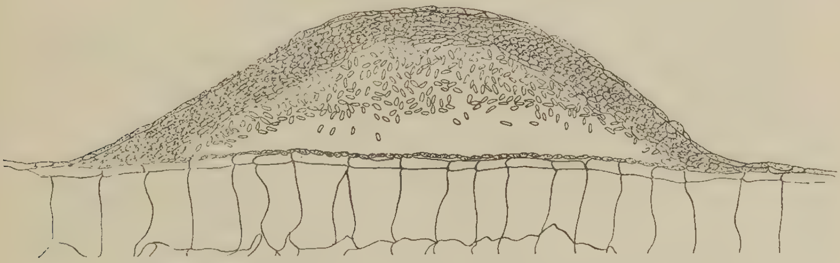


Fig. 50. *Parastigmatellina asiatica* BATISTA & COSTA n. sp. Córte longitudinal de um picnostroma subcuticular. Orig.

Sôbre folhas de *Costus* sp. associado a *Parastigmatea asiatica* BAT. & COSTA n. sp. e outros fungos. Ilha de Luzón, Filipinas. Tipo, 15855, no Herbário Bogoriense, Bogor.

Micelium liberum nullum. Pycnostromata epiphylla, cuticula tecta, sparsa, scutato-conoidea, dimidiata, 130—185 μ diam., 120—155 μ alt. pseudo-ostiolata, atro-brunnea, opaca, pariete superiore 36—48 μ cr., pseudo-parenchymatico, glabrato; hymenium inversum; pariete basali indistincto; hypostromata nulla. Pycnidiosporae sessiles, bacillares, continuae, hyalinae, 3.5—5×1 μ, in substantia mucosa evolutae. In foliis *Costi* sp. socia *Parastigmatei asiatici* Bat. & Costa, n. sp. Luzon Philipp. Typus, 15855, in Herbarium Bogoriensis-Bogor.

Stellothyriella Bat. & Ciferri n. gen.
Tipo: *S. graminis* (Wehmeyer) nobis.

Micélio livre ausente. Picnostromas subcuticulares, orbiculares, escutelar-dimidiados, radiados, marron, de dehiscência estelar. Himenio invertido. Picnidiosporos sesseis, cilindraceos ou fusoides, 1-septados, hialinos.

Typus: *S. graminis* (Wehmeyer) nobis.

Mycelium liberum nullum. Pycnostromata subcuticularia, orbiculata, scutato-dimidiata, radiata, stellatim dehiscentia, brunnea. Hymenium inversum. Pycnidiosporae sessiles, cylindraceae vel fusoideae, 1-septatae, hyalinae.

Stellothyriella graminis (Wehmeyer) Bat. & Ciferri n. comb.
Syn.: *Leptothyriella graminis* Wehmeyer
in Sydowia, VI: 436, 1952.

Micélio livre ausente. Picnidios subcuticulares, radiados, orbiculares ou angulosos, 150—300 μ de diam, pseudo-ostiolados. Himenio invertido; parede basal nula. Conidios sesseis, cilindraceos ou fusoides, hialinos, a principio continuos, depois 1-septados, 12.5—18 × 1.5—2.5 μ.

Sôbre *Festuca viridula, Muhlenbergia filiformis Phleum* sp. — Estados Unidos.

ut *Stellathyriella*

Pycnostromata subcuticularia, stellata dehiscentia, cum pycnidiosporis 1-septatis, hyalinis.

Plenotrichaceae n. fam., Bat. & Ciferri

Micélio livre peliculoso e picnostromas superficiais, marron-negros, verde-negros ou negros, escutelares, meandriformes — pletenquimáticos, nunca radiados, ostiolados ou não, de himenio invertido, sem hipostroma, e picnidiosporos de diversas formas, hialinos ou não.
Tipo: **Plenotrichum** Syd.

Mycelium liberum pelliculosum; pycnostromata superficialia, atro-brunnea, virideo-brunnea vel atra, scutata, meandrico-plectenchymatica, nec radiata, ostiolata vel non, hymenium inversum, hypostromata nulla. Pycnidiosporae variae.
Typus: **Plenotrichum** Syd.
Reunem-se nessa familia algumas fórmas imperfeitas que se associam aos Micropeltaceae.

PLENOTRICHACEAE

CHAVE PARA GÊNEROS

HIALOAMEROSPOROS

A) Micélio livre peliculoso, escasso ou ausente.
B) Picnostromas meandriformes.
C) Ostiolados.
D) Conidioforos septados, pleurogenos .. **Plectopeltis** Syd. (Ann. Myc. XXV: 125, 1927)
DD) Conidioforos ausentes. **Hansfordiopeltis** Bat. & Costa (Publ. 67, Inst. Mic. Univ. Rec. 1956)
CC) Dehiscencia estelar.
F) Picnidiosporos cilindraceos, catenulados .... **Plenocatenulis** Bat. & Cif.
BB) Picnostromas meandro-plectenquimáticos
CCC) Astomos.
D) Conidioforos ramificados, septados, fragmentaveis em picnidiosporos .... **Plenotrichum** Syd. (Ann. Myc. XXV: 131, 1927)
DD) Conidioforos ausentes .. **Plenotrichella** Bat. & Vital
AA) Micélio peliculoso, não escasso.
B) Picnostromas meandriformes; ostiolados; himenio invertido e também basal; conidioforos anastomosados, flabeliformes, acropleurogenos e unilateralmente férteis .... **Plectopycnis** Bat. & Vital
AAA) Micélio reticulado com algumas hifas cerebriformes; picnostromas meandro-plectenquimáticos; dehiscencia em fenda longitudinal; conidioforos clavados; picnidiosporos oblongos ou ovoides .... **Cribropeltis** Tehon (Myc. XXV: 252, 1933)

HIALOESCOLECOSPOROS

Micélio peliculoso, escasso ou ausente; picnostromas meandriformes; dehiscencia glebosa; picnidiosporos sesseis, multiseptatos .... **Stigmopeltis** Syd. (Ann. Myc. XXV: 127, 1927)

Plenocatenulis Batista & Cif. n. gen.
Tipo: *P. magnoliae* Bat. & Cif.

Micélio livre ausente. Picnostromas superficiais, plano-escutelares, orbiculares, de margens peliculosas, marron-olivaceos, meandriforme-plectenquimáticos, de dehiscencia-estelar; parede basal tenuissima. Himenio invertido. Conidioforos quase obsoletos. Picnidiosporos cilindraceos, continuos, catenulados, hialinos.

Typus: *P. magnoliae* Bat. & Cif.

Mycelium liberum nullum.

Pycnostromata superficialia, plano-scutata, orbiculata, marginibus pelliculosis, brunneo-olivacea, meandrico- plectenchymatica,

stellata dehiscentia efformata. Hymenio inverso. Conidiophoris quasi obsoletis. Pycnidiosporae bacillares, cylindraceae, catenulatae, hyalinae.

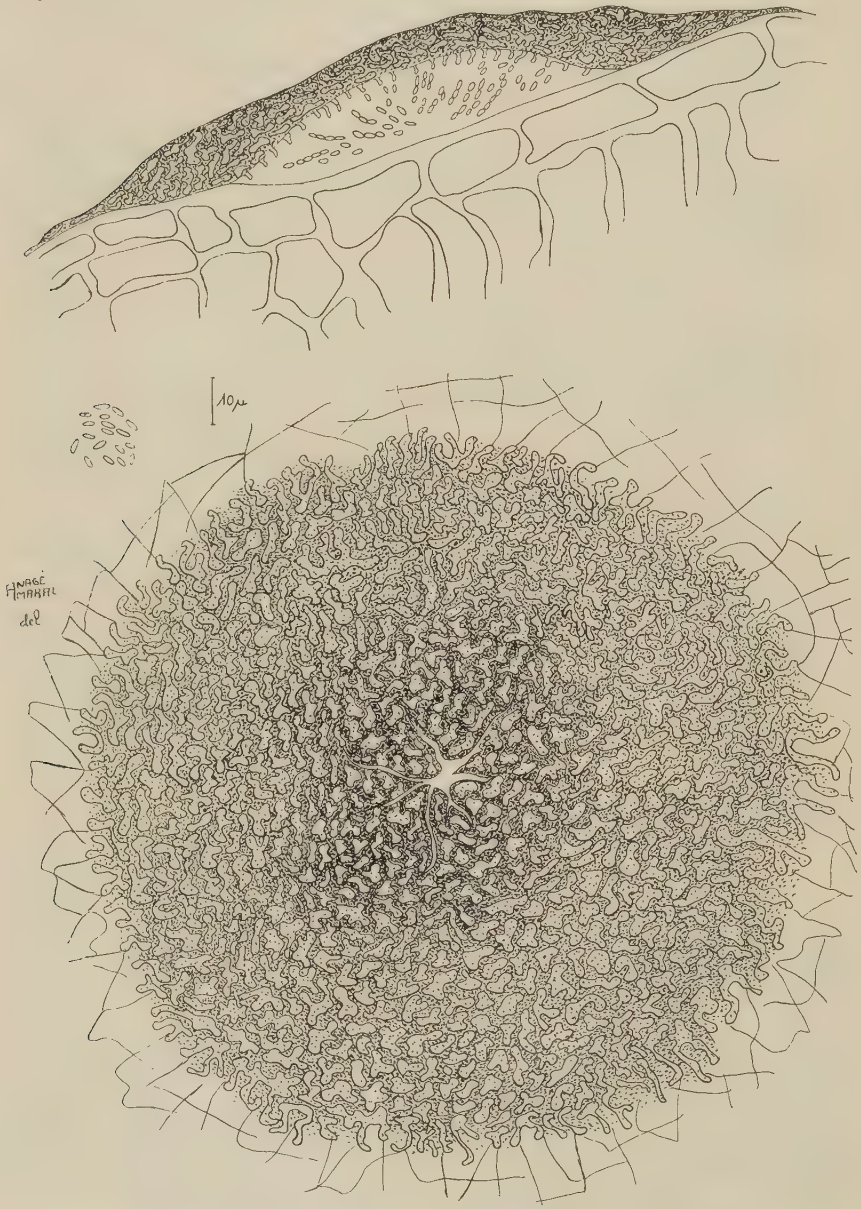


Fig. 51. *Plenocatenulis magnoliae* Batista & Ciferri
a) Picnostroma; b) picnidiosporos; c) picnostroma visto sob córte longitudinal. Orig.

Plenocatenulis magnoliae BATISTA & CIF. n. sp.

Micélio livre ausente. Picnostromas epifilos, superficiais, espalhados, plano-escutelares, orbiculares, 125—215 μ de diam, 19—25 μ de alt. marron-olivaceos, margens peliculosas, hialinas; parede superior 4.5—7.5 μ de espessura, meandriforme plectenquimática, de dehiscência estelar; parede basal tenuissima. Himenio invertido. Conidioforos cilindraceos, muito curtos, 1.5—3 × 1—2 μ, hialinos. Picnidiosporos cilindraceos, continuos, catenulados, hialinos, 2—4 × 1.5—2 μ.

Sôbre folhas de *Magnolia glauca*, associado a *Tripospermum* e *Microxyphium*. Green Cove Springs, Fla. Março, 1883, Dr. GEO MARTIN. Tipo, em ELLIS & EVERHART, 1545, sob a etiqueta de *Capnodium pelliculosum* BERK. & RAV., ex P. A. SACCARDO Herbario.

Mycelium liberum nullum. Pycnostromata epiphylla, superficialia, sparsa, plano-scutata, orbiculata, 125—215 μ diam., 19—25 μ alt., brunneo-olivacea, marginibus pelliculosis, hyalinis; pariete superiore 4.5—7.5 μ cr. meandrico- plectenchymatico, stellatim dehiscenti; pariete basali tenuissimo. Conidiophora cylindracea, multo curta, 1.5—3 × 1—2 μ, hyalina. Pycnidiosporae cylindraceae, continuae, catenulatae, hyalinae, 2—4 × 1.5—2 μ. In foliis *Magnoliae glaucae*, socia *Tripospermi* et *Microxyphii*. Green Cove Springs, Fla. 3/1883, Dr. GEO MARTIN. Typus, in ELLIS & EVERHART, North American Fungi, nº. 1545, sub *Capnodium pelliculosum* BERK. & RAV., ex P. A. SACCARDO Herbario.

Agradecemos á Universidade de Pavia, Italia, pela cessão dêsse especimen da coleção de SACCARDO.

Plenocatenulis osmanthi BAT. & CIFERRI n. sp.

Desprovido de micélio livre. Picnostromas epifilos, superficiais, dispersos, orbiculares, plano-escutelares, 160—300 μ de diam, 16.5—30 μ de altura, marron, membranosos, de dehiscência estelar; parede superior meandriforme; himenio invertido; parede basal inobservada. Conidioforos prismaticos ou cilindro-lobados, continuos, hialinos, 4.5—5.5 × 1.5—3 μ. Picnidiosporos cilindraceos, catenulados, continuos, hialinos, 4—6 × 1.5—2.5 μ.

Sôbre folhas de *Osmanthus americanus* (L.) Gray-River, N.W. of Izagora, Florida. Leg. R. K. GODFREY, 5.3.956. Tipo, 10841, Instituto de Micología, Universidade do Recife; isotipo em The National Fungus Collections, U.S.A.

Obs.: Agradecemos ao Dr. JOHN A. STEVENSON pelo envio dêsse especimen, para estudo.

Mycelium liberum nullum. Pycnostromata epiphylla, superficialia, sparsa, orbiculata, plano-scutata, 160—300 μ diam., 16.5—30 μ alt., brunnea; pariete superiore membranoso, stellatim dehiscenti; hymenium inversum; pariete basali nullo. Conidio-

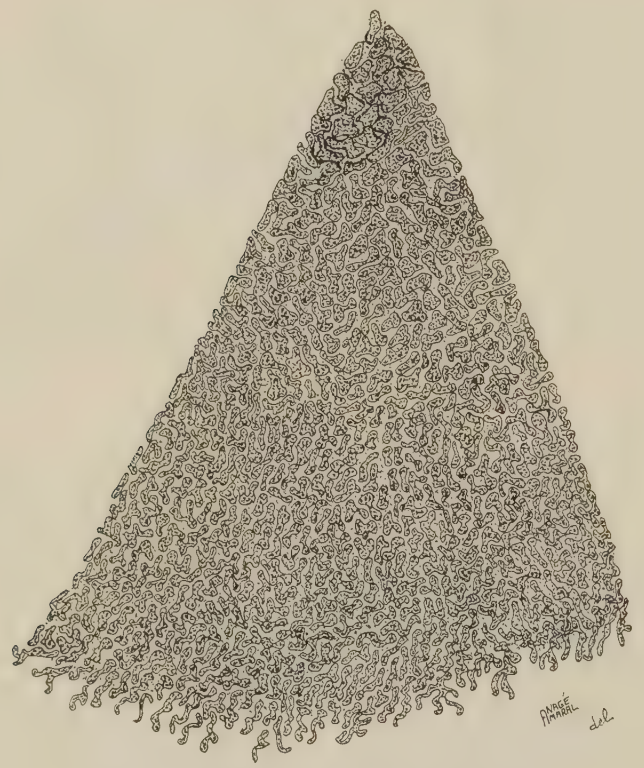


Fig. 52. *Plenocatenulis osmanthi* Batista & Cif. n. sp.
Sector da parede superior, meandriforme, de picnostroma.

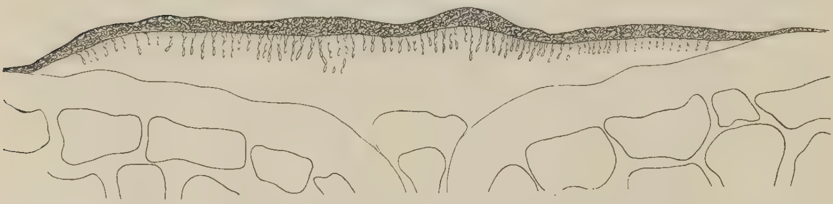


Fig. 53. *Plenocatenulis osmanthi* Batista & Cif. n. sp.
Picnostroma sob córte longitudinal.

phoris prismaticis vel cylindraceo-lobatis, continuis, hyalinis, 4.5—5.5 × 1.5—3 μ. Pycnidiosporae cylindraceae, catenulatae, continuae, hyalinae, 4—6 × 1.5—2.5 μ. In foliis *Osmanthusi americani* — River, N.W. Izagora, Fla. R. K. Godfrey, 5.3.956. Typus, 10841, Instituto de Micología, Universidade do Recife; isotypus in The National Fungus Collections, U.S.A.

Plectopycnis BATISTA & VITAL nov. gen.

Micélio peliculoso, marron, de hifas ramificadas irregularmente ou reticuladas. Picnostromas superficiais, escutelares, marron, ostiolados, meandriforme-reticulados; himênio supero e ínfero. Conidioforos flabeliformes constituidos por hifas septadas, anatomosadas, hialinas. Picnidiosporos acropleurógenos ou unilaterais, contínuos, bacilares, hialinos.
Tipo: *P. coccolobae* BAT. & VITAL.

Mycelium pelliculosum, brunneum, ex hyphis ramosis, reticulatis compositum. Pycnostromata superficialia, scutata, brunnea, ostiolata, meandrico-reticulata; hymenio supero et infero. Picnidiosporophora flabelliformia, ex hyphis septatis, hyalinis composita. Pycnidiosporae acropleurogenae vel unilaterales, bacillares, continuae, hyalinae. — Typus *P. coccolobae.*

Obs.: Êsse genero é afim de **Plenotrichum** SYD. distinguindo-se porém, por seus picnostromas ostiolados, de himenio invertido e tambem basal, com conidioforos anastomosados em estruturas flabeliformes.

Plectopycnis coccolobae BATISTA & VITAL n. sp.

Micélio peliculoso, em plágulas de 10—15 mm de diam., rotundas ou irregulares, cinza-negro, constituido por hifas marron, de 2—4 μ de diam., muito ramificadas, reticuladas. Picnostromas epífilos escutelares, orbiculares, 120—155 μ de diam., isolados, marron, de estrutura prosenquimática, reticulado-meandriforme, formada por células hifais de 1.5—2.5 μ de diam., indistintamente septadas, marron, ostíolo circular, pertuso, 5—12.5 μ de diam., e margens peliculosas. Himênio duplo, súpero e ínfero; parede superior 7.5—10 μ de diam.; parede inferior 1.5—4 μ de diam. Conidioforos flabeliformes, formados pela soldadura de hifas septadas, constrictas, 2—3 μ de diam., multicelulares, hialinas. Picnidiosporos bacilares, acropleurógenos ou unilaterais, inseridos nas áreas de septos dos picnidiosporóforos, contínuos, hialinos, 2—3 × 1—1.5 μ.
Sôbre folhas vivas de *Coccoloba paniculata* MEISSN., associado a outros fungos. Vitória. Leg. SEVERINO JOSÉ DA SILVA, 24.4.56. Tipo 5477, Instituto de Micologia, Universidade do Recife.

Mycelium pelliculosum, plagulis rotundatis vel irregularibus, atro-cinereis, 10—15 mm diam., ex hyphis brunneis, 2—4 μ diam. irregulariter ramosis vel reticulatis, compositum. Pycnidia scutata, superficialia, orbiculata, 120—155 μ diam., isolata, brunnea; ostiolo centrali pertuso, 5—12.5 μ diam.; pariete superiore prosenchymatico, meandrico-reticulato, 7.5—10 μ cr., ex cellulis 1.5—2.5 μ cr., marginibus pelliculosis, subhyalinis efformato; pariete inferiore 1.5—4 μ cr. Hymenio supero et infero. Pycnidiosporophoris flabelliformibus, 2—3 μ diam., anastomosantibus, hyalinis. Pycnidiosporae bacillares, acropleurogenae vel unilaterales, in areas

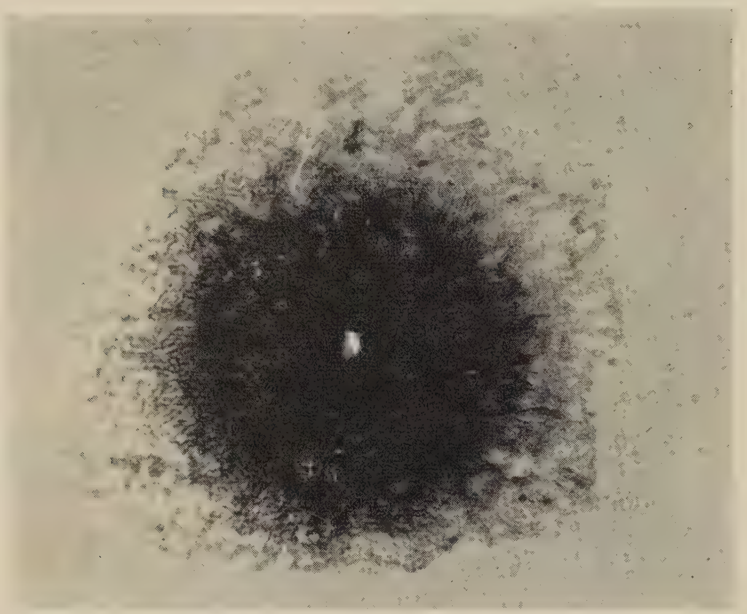


Fig. 54. *Plectopycnis coccolobae* Batista & Vital n. sp.
Picnidio fimbriado e ostiolado. Orig.

430 ×

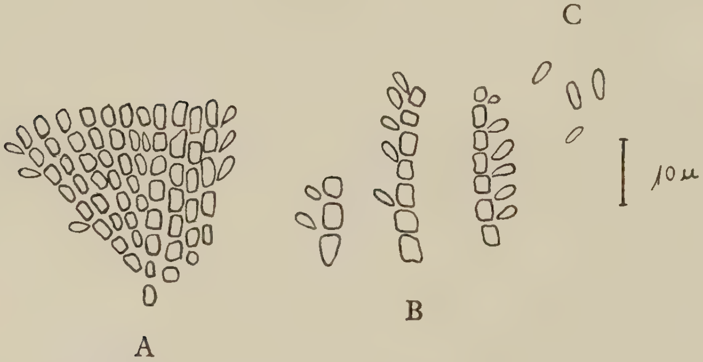


Fig. 55. *Plectopycnis coccolobae* Batista & Vital n. sp.
a) Picnidiosporoforo flabelliforme; b) picnidiosporoforos de conidios inseridos lateralmente; c) picnidiosporos. Orig.

pycnidios porophororum septatas insitae, continuae, hyalinae, 2—3 × 1—1.5 µ. — In foliis vivis *Coccolobi paniculati* cum diversis fungis. Vitoria. Leg. Severino José da Silva, 24.4.56. Typus 5477, Instituto de Micologia, Universidade do Recife, Prov. Pernambuco, Brazil, Amer. Austr.

Plenotrichella BAT. & VITAL n. gen.

Tipo: *P. perseae,* nobis.

Micélio livre tenue peliculoso, escasso, de hifas marron-negras, não setoso, e sem hifopodios.

Picnostromas superficiais, escutelares, orbiculares, astomos, glabros, marron, meandro-plectenquimaticos. Himenio invertido. Conidioforos ausentes. Picnidiosporos elipsoide-bacilares, continuos, hialinos, formados em substancia mucosa.

Typus: *P. perseae* nobis.

Mycelium liberum pelliculosum, restrictum, ex hyphis atrobrunneis, non setosum, sine hyphopodiis, compositum. Pycnostromata superficialia, scutata, orbicularia, astoma, glabrata, brunnea, meandrico-pletenchymatica. Hymenium inversum. Pycnidiosporae sessiles, ellipsoide-bacillares, continuae, hyalinae, in substancia mucosa congestae.

Obs.: O gênero em causa difere de **Plenotrichum** SYD. por não possuir conidioforos.

Plenotrichella anacardii BATISTA & COSTA n. sp.

Plágulas epifilas, irregularmente circulares, oliváceas, com pontuações negras, medindo 5—10 mm de diam. Micélio superficial, peliculoso, de hifas septadas pouco constrictas, abundantes, ramificadas, de disposição arborescente, olivaceo-marron, tendo células de 10.5—16.5 × 2.5—3 μ recobrindo os picnostromas. Ausencia

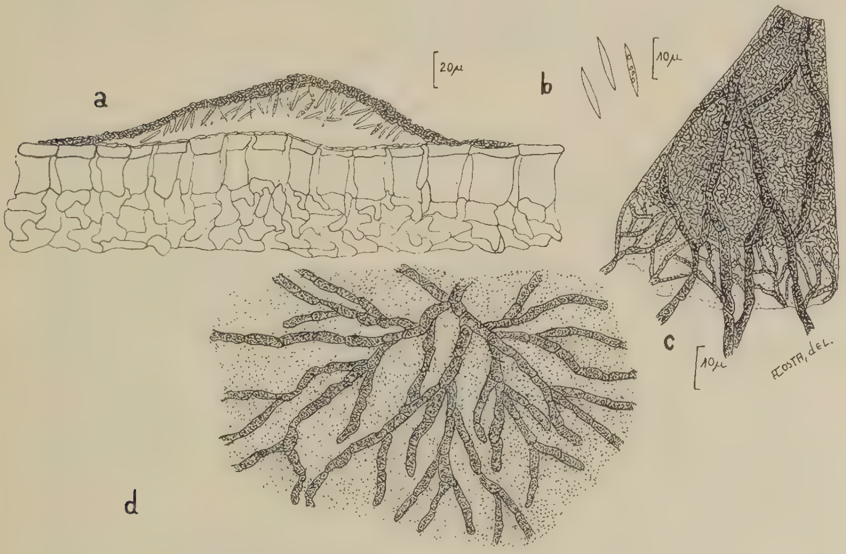


Fig. 56. *Plenotrichella anacardii* BATISTA & COSTA n. sp.
a) Córte longitudinal de picnostroma; b) picnidiosporos; c) sector da parede superior de picnostroma; d) micélio peliculoso.

de setas e hifopódios. Picnostromas superficiais, membranosos, isolados, orbiculares, escutelares, dimidiados, meandriformes, astomos, de dehiscencia irregular á maturidade, 364—510 μ de diametro, 16—22 μ de altura, marron, glabros; bordos tenues, marron-claros, peliculosos, até 97 μ de extensão; Parede inferior indistinta. Conidioforos não observados. Himenio supero, portanto invertido. Picnidiosporos fusoides, continuos, sesseis, retos ou recurvados, com 8.5—13 × 1—2.5 μ.

Sôbre folhas vivas de *Anacardium occidentale* L. associado a *Catenulaster anacardicola* BAT. & COSTA. Jaboatão-Leg. Prof. HERMANO CASTRO, 18.1.957. Tipo, 5982, Instituto de Micología, Universidade do Recife.

Plagulae epiphyllae, irregulariter rotundatae, olivaceae, 5—10 mm diam. Mycelium superficiale, pelliculosum, ex hyphis septatis, constrictis, arborescentibus, ramosis, olivaceo- brunneis, ex cellulis 10.5—16.5 × 2.5—3 μ compositum, non setosum, non hyphopodiatum. Mycelium internum et hypostromata nulla. Pycnostromata superficialia, membranosa, sparsa, orbicularia, scutato-dimidiata, 364—510 μ diam., 16—22 μ alt., brunnea, glabrata; marginibus tenuibus, brunnescentibus, pelliculosis, usque 97 μ longis; pariete superiore prosenchymatico, membranoso, 5—11 μ cr.; pariete basali inconspicuo. Hymenium inversum. Conidiophora non visa. Pycnidiosporae fusoideae, continuae, sessiles, rectae vel incurvatae, guttulatae vel non, hyalinae, 8.5—13 × 1.2—2.5 μ.

In foliis vivis *Anacardii occidentalis* L., socia *Catenulastris anacardicolae*. BAT. & COSTA. Jaboatão — Leg. Prof. HERMANO CASTRO, 18.1.1957. Typus, 5982, Instituto de Micologia, Universidade do Recife, Pernambuco, Brasil.

Plenotrichella perseae BATISTA & VITAL n. sp.

Micélio superficial, tenue, peliculoso, restricto, formado por hifas marron, septadas, constrictas, com células cilindraceas, de 5—8.5 × 2.5—4 μ; não setoso e sem hifopodios. Picnostromas superficiais, escutelares, orbiculares, glabros, 55—127 μ de diam, 20—27 μ de altura, marron, astomos, fendilhando-se irregularmente á maturidade; Himenio invertido; parede superior meandro-pletenquimática, 5—9 μ de espessura, constituida por células irregulares, 4—10 μ no maior diam.; parede basal indistinta. Conidioforos ausentes. Picnidiosporos elipsoide-bacilares, continuos, hialinos, 2—3 × 1 μ.

Sôbre ramos vivos de *Persea gratissima* GAERTN., associado a *Setopeltis perseae* BATISTA & VITAL, como fórma imperfeita. — Capunga, Recife. Leg. A. CHAVES BATISTA, 26.3.56. Tipo, 5388 — Instituto de Micologia, Universidade do Recife.

Mycelium superficiale, tenuiter pelliculosum, restrictum, ex hyphis brunneis, septatis, constrictis, ex cellulis cylindraceis, 5—8.5 × 2.5—4 μ, non setosum, non hyphopodiatum, compositum. Pycnostromata superficialia, scutata, orbicularia, glabra, 55—127 μ

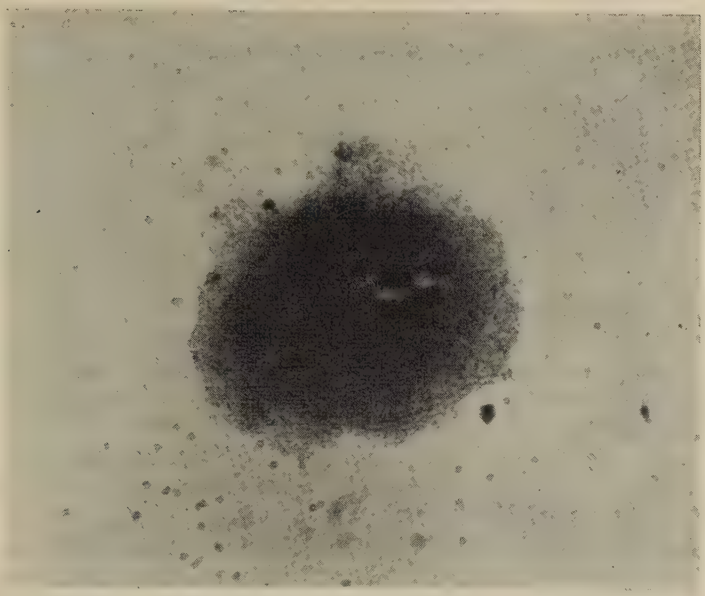


Fig. 57. *Plenotrichella perseae* BATISTA & VITAL n. sp.
Picnidio.

diam., 20—27 μ alt., brunnea, astoma; hymenium inversum; pariete superiore meandrico-plectenchymatico, 5—9 μ cr., ex cellulis irregularibus, 4—10 μ in l. diam.; pariete basali indistincto. Conidiophoris nullis. Pycnidiosporae ellipsoido-bacillares, continuae, hyalinae, 2—3 × 1 μ.

In ramulis vivis *Perseae gratissimae* GAERTN. socia *Setopeltis perseis* BAT. & VITAL. Capunga — Recife, Leg. A. CHAVES BATISTA, 26.3.56. Typus, 5388, Instituto de Micologia, Universidade do Recife, Prov. Pernambuco, Brasil, Amer. Austr.

Rhizothyriaceae TEHON emend. BAT. & CIFERRI, nobis

Picnostromas superficiais, marron-negros, privados de micélio livre, escutelares, radiados, prosenquimáticos ou plectenquimáticos, de himenio invertido, ostiolados ou não; columela central, única, ou multipla, conectada com um hipostroma ou micélio interno. Picnidiosporos de várias fórmas, hialinos ou corados.

Tipo: **Rhizothyrium** NAUMOFF.

Mycelium liberum nullum; pycnostromatis superficialibus, atrobrunneis, scutatis, radiatis vel non, ostiolatis vel non, hymenio inverso; columella centralis, una vel multipla, cum hypostromatis conexa. Pycnidiosporae variae.

Typus: **Rhizothyrium** NAUMOFF.

A essa familia, conforme já sugerido por TEHON, agregam-se algumas fórmas imperfeitas de Polystomellaceae.

RHIZOTHYRIACEAE

CHAVE PARA GÊNEROS

HIALOAMEROSPOROS

A) Micélio livre ausente; picnostromas radiados, superficiais, sem hipostroma, pluriloculares; conidioforos curtos.

B) Ostiolo presente; columela central constituida de uma única célula hialina; picnidiosporos elipsoides .... **Actinopelte** (Sacc.) Theiss. (Ann. Myc. XI: 507, 1913)

BB) Picnostromas astomos.

C) Columela central; picnidiosporos catenulados .... **Sirothyriella** v. Höhn. (Sitzb. Acad. Wien. 149: 451, 1910)

CC) Várias columelas; picnidiosporos bacilares .... **Synostomina** Petrak (Sydowia 3: 245, 1949)

AA) Hipostroma bem desenvolvido.

C) Picnostromas pluriloculares; picnidiosporos subglobosos ou elipsoides .... **Queenslandia** Bat. & Maia

CC) Picnostromas uniloculares; picnidiosporos elipsoides ou ovoides ...... **Palawaniopsis** Bat., Ciferri & Nasc.

AA) Picnostromas plectenquimáticos, não radiados; astomos; várias columelas; conidioforos lageniformes; picnidiosporos ovoides, oblongos ou bacilares .... **Columnothyrium** Bubák (Ber. Deut. Bot. Ges. 34: 308, 1916)

FEOAMEROSPOROS

A) Micélio livre ausente; picnostromas radiados; hipostroma bem desenvolvido.

B) Ostiolo presente; várias columelas; picnidiosporos elipsoides .... **Peltistroma** P. Henn. (Hedwigia, 43: 391, 1904)

BB) Astomos; várias columelas; picnidiosporos oblongo-elipsoides.

C) Loculo central fertil .... **Achoropeltis** Syd. (Ann. Myc. XXVII: 79, 1927)

CC) Loculo central estéril .... **Pycnostema** Syd. (Ann. Myc. XXV: 113, 1927)

HIALOFRAGMOSPOROS

Micélio livre ausente; picnostromas radiados; astomos; columela central; picnidiosporos cilindricos .... **Rhizothyrium** Naumoff (Bull. Soc. Myc. Fr. 30: 429, 1914)

HIALOESCOLECOSPOROS

Micélio livre ausente; picnostromas radiados; astomos; columela central; picnidiosporos recurvados .... **Actinothyrium** G. KUNZE (Myk. Heft. 2: 81, 1923)

Queenslandia n. gen. BATISTA & MAIA.
(Em homenagem ao Estado de Queensland, Australia).

Micélio livre ausente. Picnostromas superficiais, maculicolas, orbiculares, escutelares, plano-convexos, dimidiados, negros, astomos, crustáceos, radiantes, de margens subintegras; columelas varias, circundadas por um prosenquima de còr marron, e penetrando ao parenquima foliar, com hipostroma bem desenvolvido; lóculos picnidiais diversos, com himenio invertido; conidioforos lageniformes, continuos; picnidiosporos acrogenos, subglobosos a elipsoides, continuos, hialinos, estrato basal delgado, marron-claro.

Mycelium liberum nullum. Pycnostromata superficialia, maculantia, orbiculata, scutata, plano-convexa, dimidiata, atra, membranosa, ex hyphis radiantibus composita, marginibus subintegris; columellae diversae in prosenchymo brunneo evolutae, in parenchyma foliorum immersae cum hypostromata bene definita; loculis pycnidialibus diversis; hymenio inverso; pariete basali tenui, brunneio efformata. Condiophoris continuis, lageniformibus. Pycnidiosporae acrogenae, subglobosae vel ellipsoideae, continuae, hyalinae.
Nota: Êste gênero distingue-se de **Synostomina** PETRAK pelos picnostromas maculicolas, com varias columelas originadas de amplo prosenquima e que penetram ao parenquima foliar produzindo hipostroma bem definido, tendo ainda picnidiosporos, subglobosos ou elipsoides.
Difere ainda de **Columnothyrium** BUBÁK pela natureza radiada da parede superior dos picnostromas.

Queenslandia pulchella BATISTA & MAIA n. sp.

Micélio livre ausente. Picnostromas superficiais, epífilos, orbiculares, escutelares, plano-convexos, 1—1.5 × 0.5—1 mm, crustáceos, negros, astomos, manchando o tecido foliar com um halo marron, isolados ou confluentes, radiantes, de margens subintegras; parede superior 8—10 μ de espessura, constituida por células retangulares, 8—10 × 3.5—5.5 μ. Sob a parede inferior do picnostroma em vários pontos, desenvolve-se um prosenquima de côr marron que origina columelas de 34—40 × 34—38 μ as quais penetram o mesófilo do hospedeiro, formando bem definido hipostroma e micélio interno hialino, ramificado, com hifas septadas de 2—2.5 μ diam. Em tôrno das columelas situam-se os lóculos picnidiais, de 340—610 μ de diam, 21—32 μ de alt. os quais apresentam o himenio invertido, tendo conidioforos lageniformes, continuos, hialinos, 8—11 × 1—2 μ. Picnidiosporos acrogenos, subglobosos a elipsoides, continuos, hialinos, 2.5—3 × 1—1.5 μ. Estrato basal delicado, marron - claro, 0.6—1.5 μ de espessura.

Sôbre folhas de *Alsophila rebecca* N. E. Queensland, Australia, Leg. MUELLER, Tipo, em Melbourne Botanic Gardens and National Herbarium, South Yarra, Australia.

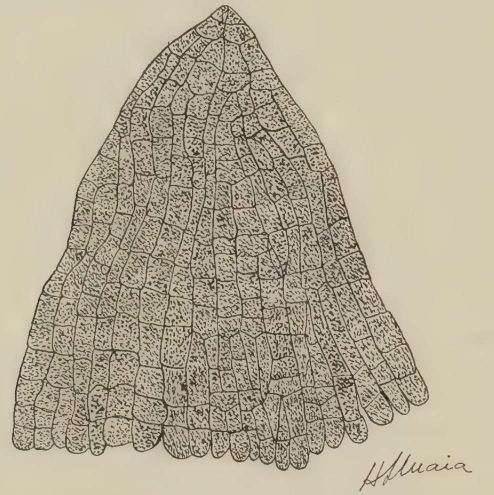


Fig. 58. *Queenslandia pulchella* BATISTA & MAIA n. sp.
Sector do picnostroma.

Obs.: O fungo em tela foi-nos cedido gentilmente pelo Dr. A. W. JESSEP da aludida Instituição da Austrália.

O especimen é parte do tipo de *Asterina alsophilae* CKE. & MASS. que THEISSEN (Ann. Mycol. X: 7, 1912 e ibidem XIII: 246, 1915) reconheceu como *Polystomella pulchella* (SPEG.) THEISSEN, em virtude de sua identidade com o *Microthyrium pulchellum* SPEG. (F. Guar. 1. no 295).

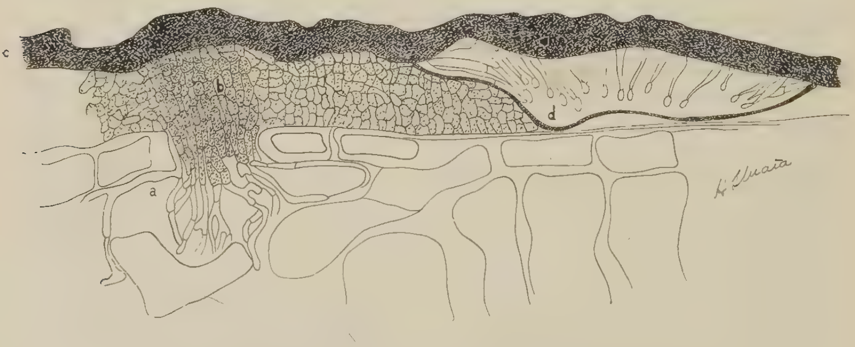


Fig. 59. *Queenslandia pulchella* BATISTA & MAIA n. sp.
Picnostroma sob córte longitudinal.
a) Hipostroma; b) columela; c) parede superior; d) lóculo picnidial, de himenio invertido.

Não assinalamos a presença de ascostromas, nem de ascos, consequentemente, de modo que descrevemos a especie em apreço, situando-a na família Rhizothyriaceae. É provável, por outro lado, que se trate da fórma imperfeita do *Polystomella* estudado por THEISSEN noutra fração do tipo.

Micelium liberum nullum. Pycnostromata superficialia, epiphylla, orbiculata, scutata, plano-convexa, 1—1.5 × 0.5—1 mm diam. crustacea, atra, in area brunneo-decolorata, simplicia vel confluentia marginibus sub-integris, astoma, pariete superiore radiato, 8—10 μ cr., ex cellulis rectangularibus, 8—10 × 3.5—5.5 μ composito; pariete basali tenui, brunnescenti, 0.6—1.6 μ cr. efformata, Columellae variae, 34—40 × 34—38 μ, in prosenchymo brunneo evolutae, penetrantes usque mesophyllum; hypostromata definita, ex hyphis intramatricalibus hyalinis, 2—2.5 μ cr., composita; loculis pycnidialibus 340—610 μ diam., 21—32 μ alt., hymenio inverso, efformatis. Conidiophoris lageniformibus, continuis, hyalinis, 8—11 × 1—2 μ. Pycnidiosporae acrogenae, simplices, subglobosae vel ellipsoideae, continuae, hyalinae, 2.5—3 × 1.5 μ.
In foliis *Alsophili rebecci* — N. E. Queensland, Australia, Leg. MUELLER. Typus, (ut nom. *Asterina alsophilae* CKE. & MASS.) — Melbourne Botanic Gardens and National Herbarium, South Yarra, Australia.

Palawaniopsis BAT., CIFERRI & NASC. n. gen.

Micélio livre ausente. Picnostromas superficiais, irregularmente orbiculares, marron-negros, radiados, crustaceos, uniloculares, de dehiscencia irregular ou longitudinal. Himenio invertido. Conidioforos lageniformes, hialinos. Picnidiosporos elipsoides ou ovoides, continuos, hialinos. Hipostroma epidermal, oblongo, multiplo, marron, conectado com as paredes basal e superior do picnostroma por meio de filamentos hifais, subhialinos ou marron.
Tipo: *P. dovyalidis* nobis.

Mycelium liberum nullum. Pycnostromata superficialia, irregulariter orbiculata, atro-brunnea, radiata, crustacea, uniloculata, irregulariter vel longitudinaliter dehiscentia. Conidiophoris lageniformibus, hyalinis. Pycnidiosporae ellipsoideae vel ovoideae, continuae, hyalinae. Hypostromata epidermalia, oblonga, multipla, brunnea, ad superficiem interiorem strati tegentis et strati basalis per diversa fila hyphalia conexa.
Typus: *P. dovyalidis* nobis.

Palawaniopsis dovyalidis BAT., CIFERRI & NASC., n. sp.

Micélio livre ausente. Picnostromas superficiais, epífilos, irregularmente orbiculares, ou semi-orbiculares, plano-convexos, marron-negros, densamente associados com os ascostromas de *Palawania dovyalidis*, tornando-se assim quase indistintos; isolados ou confluentes, dispostos em círculos concêntricos, formando colônias circulares de 2—7 mm de diam., carbonáceos, de 176—330 × 175—220 μ; altura: 41.5—50 μ na parte central; ástomos, de dehiscência irregularmente estelar ou abrindo-se em fenda longitudinal; parede superior sub-parenquimática de células retangulares ou poligonais, pouco distintas, radialmente dispostas, de 4.5—9 × 2.5—5.5 μ; bordos marron, íntegros, formados por células irregulares ou retangulares, de 7.5—16.5 × 5—9 μ; espessura da parede superior: 12—17 μ; parede tênue, hialina. Himênio invertido e lóculo picnidial único. Hipostroma epidermal, ocupando toda a célula epidérmica, múltiplo, oblongo, e formado por células poligonais marron-claras, e hialinas nas regiões mais profundas, de 3.5—9 × 2.5—4.5 μ; o estroma prende-se por numerosas hifas marron-claras a hialinas, de 1—1.5 μ de diam., septadas à face interna da parede superior do picnostroma e ao estrato basal. Conidióforos lageniformes, contínuos, hialinos, 5.5—7 × 3—4 μ. Picnidiosporos

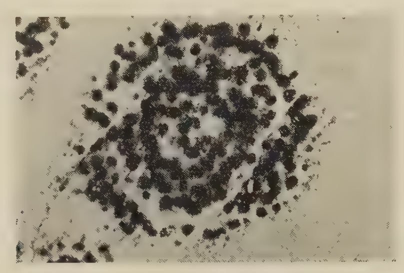


Fig. 60. *Palawaniopsis Dovyalidis* Bat. & Nasc. n. sp.
Arranjo dos picnostromas, achando-se presentes tambem alguns ascostromas.

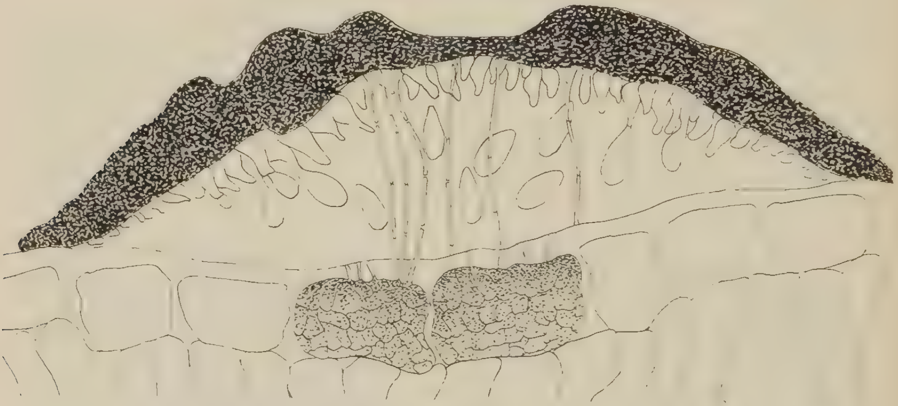


Fig. 61. *Palawaniopsis dovyalidis* Bat. & Nasc. n. sp.
a) Picnostroma sob córte longitudinal deixando ver o detalhe da constituição do hipostroma; b) picnidiosporos.

elipsoides ou ovoides, hialinos, contínuos, numerosos, 10—13.5 × 5.5—7 μ.

Sôbre folhas de *Dovyalis rhamnoides* Wilderness, George District Cape, 10.5.1923. Leg. E. M. Doidge, nº 17117. Tipo em Union of South Africa, Dep. Agric. Mycol. Herb. Está intimamente associado a *Palawania dovyalidis* (Doidge) Nel.

Mycelium liberum nullum. Coloniae epiphyllae, superficiales, irregulariter orbiculatae, 2—7 μ diam. Pycnostromata isolata vel confluentia, 176—330 × 175—220 μ, atro-brunnea, crustacea, plano-convexa, 41.5—50 μ alt., uniloculata, irregulariter stellata vel longitudinaliter dehiscentia; pariete superiore radiato, 12—17 μ cr., subparenchymatico, ex cellulis rectangularibus et polygonalibus, 4.5—9 × 2.5—5.5 μ composito; marginibus integris; pariete inferiore tenui, hyalino. Hymenium inversum. Hypostromata epidermalia, multipla, oblonga, ex cellulis polygonalibus, brunnescentibus, 3.5—9 × 2.5—4.5 μ, ad superficiem interiorem strati tegentis et strati basalis per diversa fila hyphalia conexa, brunnescentibus vel hyalinis, 1—1.5 μ cr., composita. Conidiophoris lageniformibus, continuis, hyalinis, 5.5—7 × 3—4 μ. Pycnidiosporae ellipsoideae vel ovoideae, continuae, hyalinae, 10—13.5 × 5.5—7 μ. In foliis *Dovyalidis rhamnoidis* Wilderness, George District, Cape. Leg. E. M. Doidge, 10.5.1923. Typus nº 17117, Union of South Africa, Dep. Agric. Mycol. Herb. 16.5.1957.

Peltistroma juruanum P. Henn.
in Hedwigia, 43: 391, 1904
v. Höhnel: Fragm. Zur Mykolog. XIII, nº 636, 1910
Arnaud *in* Ann. École National Agric. Montp. XVI: 216, 1918.

Micélio livre nulo. Picnostromas hipofilos, negros, plano-convexos, arredondados, 4—7 mm de diam, 27—54 μ alt., celulosicos, pluriloculares, bordos inteiros, marron-negros; parede-superior constituida por varios estratos, sendo os inferiores pseudo-parenquimaticos,

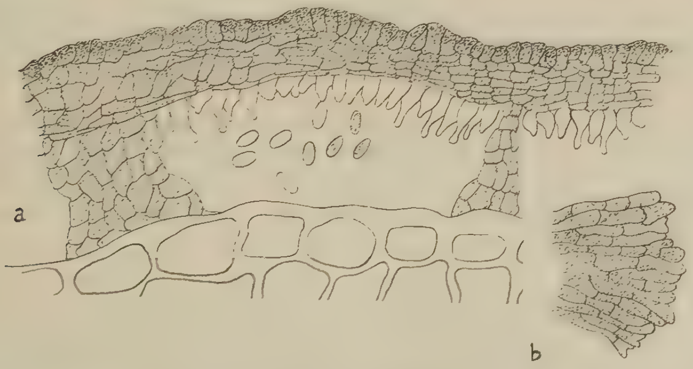


Fig. 62. *Peltistroma juruanum* P. Henn.
a) Picnostroma, sob córte longitudinal; b) margem da parede superior do picnostroma.

formados por células arredondadas de 2.5—5 μ de diam., e os superiores, prosenquimaticos, compostos por hifas radiadas de 3—12.5 × 2.5—5 μ, isto na porção central pois a região periferica é marron, menos espessa e apresenta apenas tecido de hifas radiadas; ostiolo arredondado, 5—7.5 μ de diam.; a parede que fica acima dos loculos mede de 10—25 μ e a inferior, ausente ou pouco desenvolvida mede de 35—5 μ de espessura; loculos simples ou multiplos, globoso-depressos, irregularmente difundidos no seio do estroma, 50—141 μ de diametro e 17.5—32 μ de altura; himenio invertido. Hipostroma multiplo, irregularmente disposto, constituido por um pilar cilindraceo, 25—32.5 μ de altura e 22.5—30 μ de largura, epidermico, hialino, do qual partem ramificações hifais tambem hialinas, que se desenvolvem inter e intracelularmente. Conidioforos revestindo a parede superior dos lóculos, conoides, 7—11 × 3.5—7 μ, hialinos. Conidios elipsoides, hialinos depois marron-claros, continuos, 5—7.5 × 2—3 μ, numerosos.

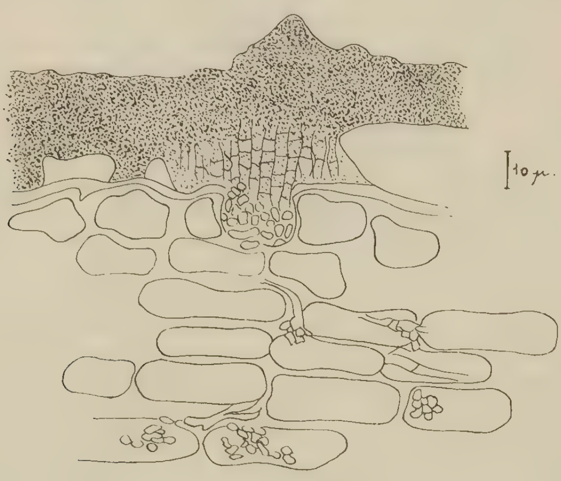


Fig. 63. *Peltistroma juruanum* P. Henn.
Detalhe da consituição do hipostroma. Orig.

Sôbre folha de hospedeiro não identificado. Ex ,,Fungi Cubenses Wrigthtiani'', nº 716, sob a etiqueta de *Rhytisma atramentarium* B. & C.

O material em tela foi-nos cedido para estudo pela gentileza do Mus. Bot. Stockholm.

Peltistroma pterocelastri Batista & Vital, n. sp.

Micelio livre ausente. Picnostromas superficiais, anfigenos, mas predominantemente epifilos, orbiculares, plano-convexos, 220—250 μ de diam., esparsos ou confluentes, circinados, marron-negros, membranoso-carbonacéos, radiados de margens um pouco fimbriadas, compostas por hifas de 1.5—3 μ de diam, pseudo-ostiolados; membrana basal marron-clara, de 1—10 μ de diam. Columelas prosenquimáticas, marron, conectadas com hipostroma colunar, 20—50 μ de diam, multiplo, bem desenvolvido, subepidermal, do qual se desenvolve micelio interno até ao tecido lacunoso; um ou vários lóculos são diferenciados por essas columelas. Os lóculos apresentam-se subglobosos ou irregulares, 75—130 μ de diam, 22—80 μ de altura, tendo a parede superior de 7.5—25 μ de alt. Himenio invertido. Conidioforos lageniformes, 1—3 μ de extensão, hialinos. Picnidiosporos elipsoides, continuos, marron-claros, 2.5—4 × 0.5—2 μ.

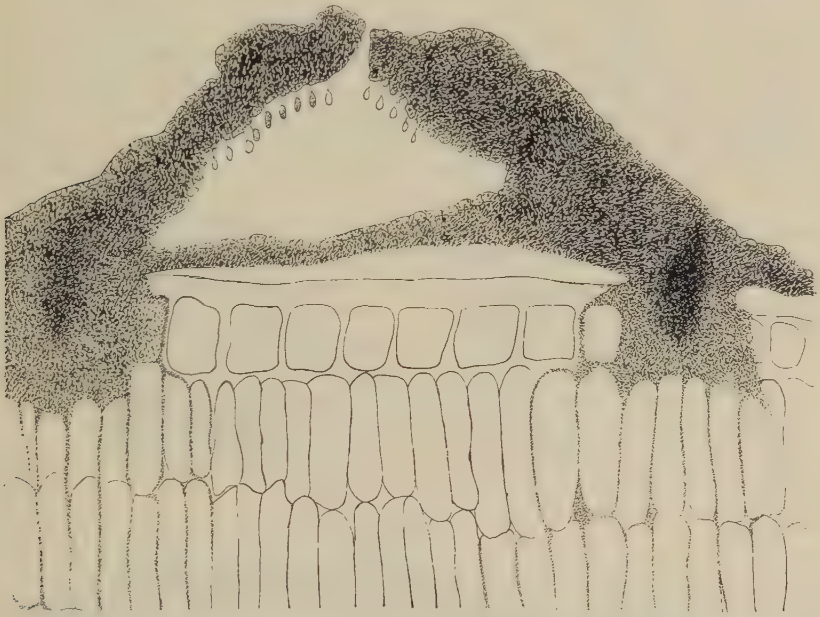


Fig. 64. *Peltistroma pterocelastri* Bat. & Vital n. sp.
Picnostromata and hypostroma.

Sôbre folhas de *Pterocelastrus galpini* Loes., associado a *Polyrhizon pterocelastri* Doidge. Kromrivier, Rustenburg District, Cape, Leg. E. M. Doidge & A. M. Bottomley, 14/12/938. Tipo, 32776, Union of South Africa, Dep. of Agric Mycol. Herb.

Mycelium liberum nullum. Pycnostromata superficialia, amphigena vel epiphylla, orbiculata, plano-convexa, 220—250 μ diam., sparsa vel confluentia, circinata, membrano-carbonacea, radiata, ex hyphis 1.5—3 μ diam., composita; marginibus frimbriatis, pseudo-ostiolata; pariete basali brunnescenti, 1—10 μ cr. Collumellae prosenchymaticae, brunneae, 20—50 μ diam., multiplae, in connexione cum hypostromatis subepidermalibus, bene evolutis.

Locula una vel plura, subglobosa vel irregularia, 75—130 μ diam., 22—80 μ alt., pariete superiore 7.5—25 μ alt.; hymenium inversum; conidiophoris lageniformibus, hyalinis, 1—3 μ long. compositis. Pycnidiosporae, continuae, brunnescentes, 2.5—4 × 0.5—2 μ.

In foliis *Pterocelastri galpini* Loes., socium, *Polyrhizon pterocelastri* Doidge. Kromrivier, Rustenburg, Distr. Cape; Leg. E. M. Doidge & A. M. Bottomley, 14/12/938. Typus, 32778, Union of South Africa, Dept. Agric. Mycol. Herb.

Obs.: Agradecemos ao estagiário Dr. J. Americo de Lima pela ajuda que nos prestou no curso dêste trabalho.

Actinothyrium smilacis Bat. & Nasc., n. sp.

Micélio livre ausente. Picnostromas superficiais, marron-negros, orbiculares, convexos, 330—680 μ de diam., 61—73.5 μ de alt., isolados ou confluentes, glabros, coriáceos, ástomos, sub-parenquimáticos, margens crenuladas, parede superior em várias camadas de células, sendo a externa constituida por células retangulares, 6—13 × 4—5.5 μ, irregularmente radiadas. Columela central ou excêntrica, marron-clara, prosenquimática, 8—10 μ de diam. Himênio invertido. Membrana basal subhialina ou marron-clara, formada por células poligonais, 3.5—7 × 2.5—5.5 μ. Micélio interno hialino, de hifas septadas, 1—2.5 μ diam., inter e intracelular, em conexão com a membrana basal do picnostroma. Conidióforos conoides ou prismáticos, contínuos, hialinos, 8.5—13 × 1.5—2.5 μ.

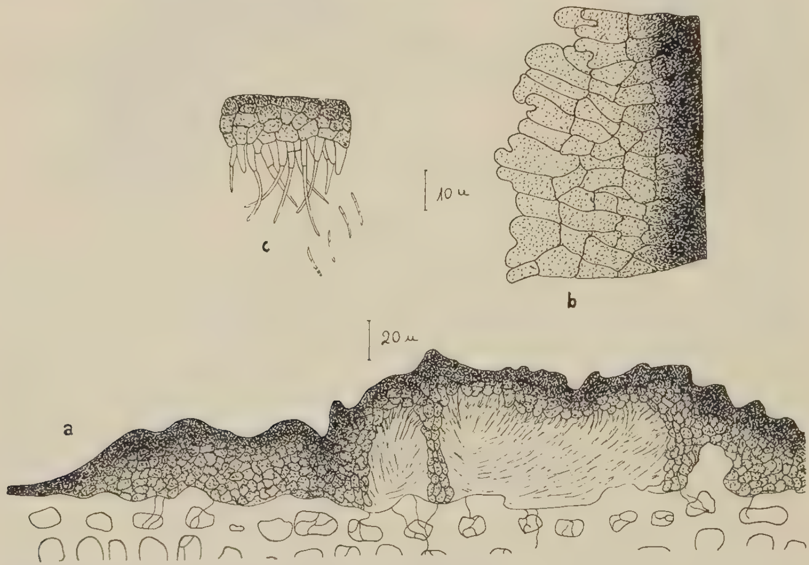


Fig. 65. *Actinothyrium smilacis* Bat. & Nasc.
a) Picnostroma sob córte longitudinal; b) margem do picnostroma; c) porção do himenio, conidioforos e picnidiosporos.

Picnidiosporos acrógenos, filiformes, septados, encurvados, hialinos, 9—10 × 0.8—1 μ.

Sôbre caule de *Smilax rotundifolii*. Newfield, New Jersey, Leg. J. B. ELLIS, Abril, 1877. Tipo nº 1448, National Herbarium of Victoria, Australia.

Nota: O especimen em tela achava-se etiquetado sob o nome de *Microthyrium smilacis* DE NOT. (Atti Acad. Torino, vol. VII, 1845). Êsse nome corresponde agora a *Peltella smilacis* (DE NOT.) STEV. (The Microthyriaceae, The Univ. Illinois Press, 1939). Contudo, não encontramos ascostromas. É provável que a espécie ora descrita constitua o estado imperfeito de *Peltella*, como tal admitido por outros autores, porém, aparentemente, não descrito.

Mycelium liberum nullum. Pycnostromata superficialia, atrobrunnea, orbiculata, convexa, 330—680 μ diam., 61—73 μ alt. sparsa vel confluentia, glabrata, coriacea, astoma; pariete superiore pluristratoso, subparenchymatico, ex cellulis rectangularibus, 6—13 × 4—5.5 μ, irregulariter radiatis constituto, marginibus crenulatis; columella centralis vel excentrica, brunnescens, prosenchymatica, 8—10 μ diam.; hymenium inversum; membrana basali subhyalina vel brunnescenti, ex cellulis polygonalibus, 3.5—7 × 2.5—5.5 μ, efformata. Mycelium internum hyalinum, intra-et intercellulae, ex hyphis septatis, 1—2.5 μ cr. in pycnostromatis membrana basalis oriunda, compositum. Conidiophoris conoideis vel prismaticis, continuis, hyalinis, 8.5—13 × 1.5—2.5 μ. Pycnidiosporae acrogenae, filiformes, septatae, incurvatae, hyalinae, 9—10 × 0.8—1 μ. — In ramulis *Smilacis rotundifolii*. Newfield, New Jersey, Leg. J. B. ELLIS, 4, 1872. Typus nº 1448, National Herbarium of Victoria, Australia.

Trichopeltulaceae n. fam. BAT. & CIFERRI.

Caracterizada por micélio superficial, marron-laminar, radiante, de hifas radiantes ou paralelas, anastomosadas, nunca livres, em forma de fita, rotundo, ou estelar-pseudopodal, prosenquimático, por picnose originando picnostromas de himenio invertido. Micélio interno raramente presente; picnidiosporos de diferentes fórmas, hialinos ou escuros.

Tipo **Trichopeltulum** SPEG.

Mycelium superficiale, brunneum, laminatum, vittaeforme, rotundatum vel stellato-amaeboidale, prosenchymaticum, ex hyphis radiatis vel parallelis, nec liberis, prosenchymaticum compositum; mycelium internum presens vel non. Pycnostromata a lamina myceliana tecta, per pycnosim evoluta. Hymenium inversum; pycnidiosporae variae.

Essa familia que corresponderia a Peltopycnidiaceae v. HÖHN, comporta algumas fórmas imperfeitas dos Trichopeltaceae, e, possivelmente, também de Trichothyriaceae.

TRICHOPELTULACEAE

CHAVE PARA GÊNEROS

HIALOAMEROSPOROS

A) Lâmina micelica semelhante a fita, marron, hifas radiadas ou paralelas.

B) Lâmina superficial, glabra, dendritica; picnostromas plano-escutelares; pseudo-ostiolados; conidioforos curtos; picnidiosporos cilindraceos ou bacilares .... **Trichopeltulum** Speg. (Fung. Puigg. p. 225, n^0 432, 1889)

BB) Lâmina superficial, glabra, simples ou ramificada, nunca dendritica; picnostromas plano-escutelares; astomos; conidioforos ausentes; picnidiosporos elipsoides .... **Pycnidiopeltis** Bat. & Costa

BBB) Lâmina superficial, simples ou ramificada, com escassas setas acuminadas, dendritica ou não, micélio interno hialino; picnostromas plano-escutelares, raramente setosos; pseudo-ostiolados; conidioforos filiformes; picnidiosporos bacilares .... **Pycnothyriella** Bat. (Bol. Sec: Agr. I. C. Pern. XIX: 107, 1952)

AA) Lâmina micelica estelar ou de margens dentadas, pseudopodal, hifas radiadas e paralelas; picnostromas convexos, salientes; pseudo-ostioladas; conidioforos curtos; picnidiosporos fusoides .... **Stellopeltis** Bat. & Vital

AAA) Lâmina micelica efusa; picnostromas globosos ou elipsoides; dehiscencia irregular; conidioforos ausentes; picnidiosporos bacilares .... **Brefeldiopycnis** Petrak & Ciferri (Ann. Myc. XXX: 267, 1932)

AAAA) Lâmina micelica arredondada, radiante; pseudoostiolados; conidioforos ausentes; picnidiosporos lineares .... **Enthallopycnidium** Stev. (Bernice P. Bish. Mus. Bul. 19: 85, 1925)

HIALOESCOLECOSPOROS

A) Lâmina micelica arredondada, lisa, radiante; superficial, plana; picnostromas globoso-deprimidos.

B) Pseudoostiolados; picnidiosporos continuos .... **Ischnostroma** Syd. (Philipp. Journ. Sc. 9: 186, 1914)

AA) Lâmina micelica verrugosa; picnostromas subglobosos; astomos; conidioforos deciduos; picnidiosporos continuos .... **Trachythyriolum** Speg. (Bol. Acad. Cord. 25: 523, 1919)

Pycnidiopeltis BATISTA & COSTA n. gen.
Typus: *P. smilacinum* nobis.

Micélio laminar, superficial, marron, radiante, prosenquimático, como fita alongada, simples ou ramificado, porém não dentritico, de margens lobadas, inteiras; hifas anastomosadas, nunca livres, sem setas ou hifopodios. Picnostromas desenvolvidos sob a membrana miceliana, por picnose, orbiculares, escutelares, dimidiados, glabros, astomos, uniloculares, membranosos; himenio invertido. Picnidiosporos sesseis, continuos, hialinos.
Typus: *P. smilacinum*, nobis.

Mycelium superficiale, laminatum, vittae-forme, brunneum, radiatum, simplex vel ramosum, non dentriticum, marginibus lobatis, integris ex hyphis anastomosantibus, non liberis, non setosis, nec hyphopodiatis, compositum. Pycnostromata a membrana mycelica tecta, pycnosica efformata, orbicularia, scutato-dimidiata, glabra, astoma, uniloculata, membranosa. Hymenium inversum. Pycnidiosporae sessiles, continuae, hyalinae.

Obs.: Éste gênero que ora propomos afigura-se-nos distinto de **Trichopeltulum** SPEG. por seu micélio laminar não dendritico-ramificado, tendo ainda picnostromas astomos, com himenio invertido. Veja-se O. PETRAK (Ann. Myc. XXXIII, pag. 173—174, 1935).

Pycnidiopeltis smilacinum BATISTA & COSTA n. sp.

Micélio epifilo, superficial, marron, laminar, radiante, prosenquimático, como fita alongada, simples ou ramificada, nunca dentritico, margens lobadas, inteiras, hifas anastomosadas, nunca livres, septadas, com células cilindricas, 6—11 × 3.5—4 μ, sem setas ou hifopodios. Picnostromas numerosos, formados por picnose sob a membrana miceliana, orbiculares, escutelares, dimidiados, 54—165 μ de diam, 16—38 μ de altura, glabros, astomos, uniloculares, membranosos; a parede superior é 5.5—19 μ de espessura; a parede inferior é indistinta. Himenio invertido. Picnidiosporos elipsoides, continuos, sesseis, hialinos, 3.5—5 × 1—1.5 μ, produzidos em substancia mucosa.

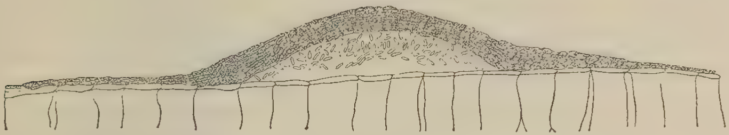


Fig. 66. *Pycnidiopeltis smilacinum* BATISTA & COSTA n. sp.
Córte longitudinal de um picnotirio com picnidiosporos originados sôbre himenio invertido. Orig.

Sôbre folhas de *Smilax* sp. associado a *Plochmopeltidella smilacina* MENDOZA and *Hormiscium* sp. Olympus, Ohau, June 24, 1921. Leg. F. L. STEVENS. Tipo, 6668. Herb. Univ. Illinois.

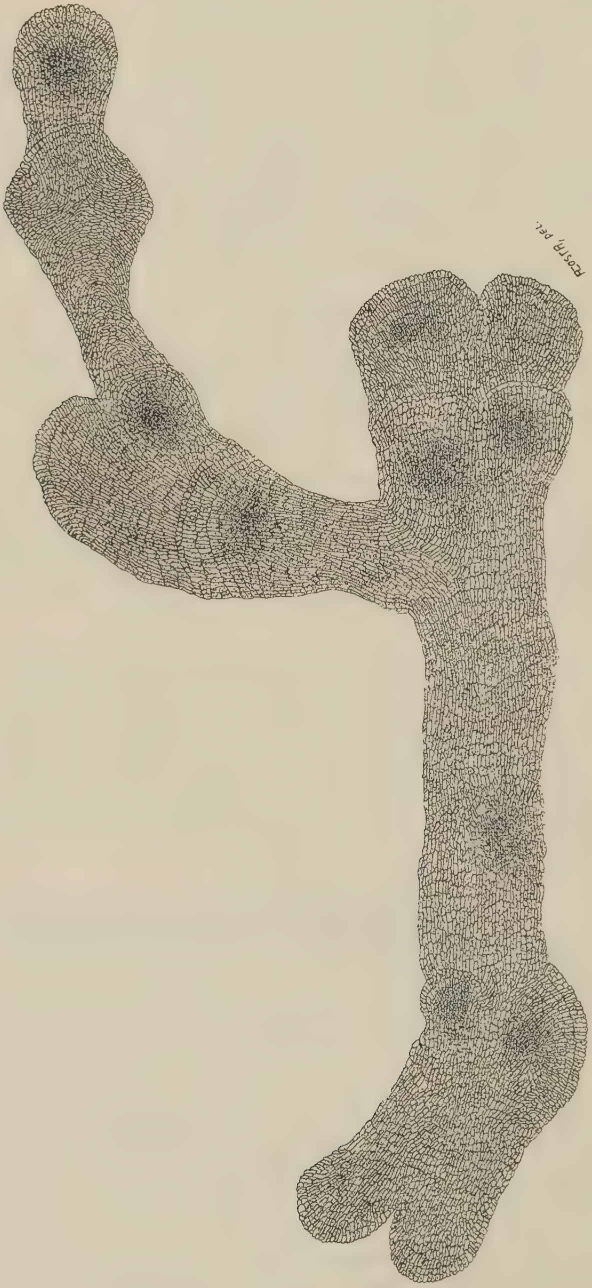


Fig. 67. *Pycnidiopeltis smilacinum* BATISTA & COSTA n. sp. Micélio laminar recobrindo picnotirios. Orig.

Ésse especimen foi-nos cedido pela cortesia de The Herbarium University of Illinois e achava-se etiquetado sob n⁰. 6668 *Phragmocapnias smilacina* MENDOZA *Plochmopeltidella smilacina* MENDOZA, „on *Pelea* sp. Evidently the host is *Smilax* sp. not *Pelea* sp."

Mycelium epiphyllum, superficiale, brunneum, laminatum, vittae forme, radiatum, prosenchymaticum, simplex vel ramosum, non dentriticum, marginibus lobatis, integris, ex hyphis anastomosantibus, nec liberis, septatis, ex cellulis cylindraceis, 6—11 × 3.5—4 μ, non setosis, nec hyphopodiatis, compositum. Pycnostromata numerosa, a membrana mycelica tecta, pycnosice efformata, orbicularia, scutato-dimidiata, 54—165 μ diam., 16—38 μ alt., glabrata, astoma, uniloculata, membranosa; pariete superiore 5.5—19 μ cr., pariete basali indistincte composito. Hymenium inversum. Pycnidiosporae ellipsoideae, continuae, sessiles, hyalinae, 3.5—5 × 1—1.5 μ, in substantia mucosa evolutae.

In foliis *Smilacis* sp. Olympus, Ohau, 24.6.921, Leg. F. L. STEVENS Typus, 6668, Herbarium University of Illinois.

Igualmente assinalado sôbre folhas de *Chrysophyllum* sp. coll. por JAMES R. WEIR, 17.8.923, Amazon River, Pará, Brasil. Ésse especimen, da coleção „Fungi of the Amazon River Drainage, n⁰ 613," foi-nos enviado para estudo pelo Dr. JOHN A. STEVENSON, The Fungus National Collections, U.S.A.

Pycnidiopeltis smilacinum var. *hymenaeae* BAT. & COSTA n. var.

Micélio superficial, epífilo, lamelar, de margens inteiras, uniestratificado, alongado, com ramificações digitiformes, marron, 1—3.5 mm de extensão, formado por hifas anastomosadas, paralelas, de células cilindráceas, 11—16.5 × 2.5—5.5 μ nunca livres; setas e hifopódios ausentes. Picnostromas desenvolvidos por picnose sob a lamina miceliana, membranáceos, prosenquimáticas, orbiculares, dimidiados, 67.5—85 μ de diam, 11—16 μ de altura, marron, uniloculares, glabros, himenio invertido, pseudo-ostiolados, parede superior radiada, 3—5.5 μ de diam., parede inferior indistinta. Picnidiosporos bacilares, continuos, retos, sesseis, hialinos, 5.5—7 × 1—1.5 μ, reunidos por uma substância mucoide; conidioforos ausentes.

Sôbre folhas de *Hymenaea microphylla* B. RODR. Beberibe, Recife, Leg. Dr. EPAMINONDAS DE BARROS CORREIA, 3-12-56. Tipo, 5933, Instituto de Micología, da Universidade do Recife.

Mycelio ex hyphis more elongatis composito, pycnostromatibus minoribus et pycnidiosporis maioribus a typo differt.

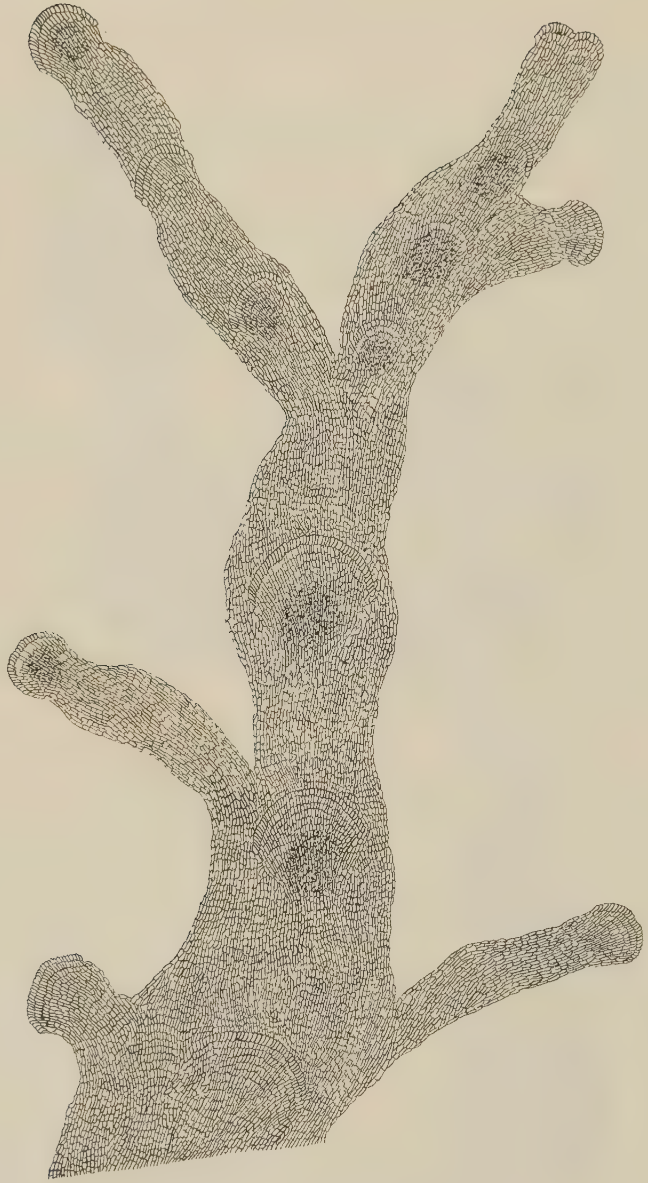


Fig. 68. *Pycnidiopeltis smilacinum* var. *hymenaeae* BAT. & COSTA n. var. Micélio laminar e picnostromas.

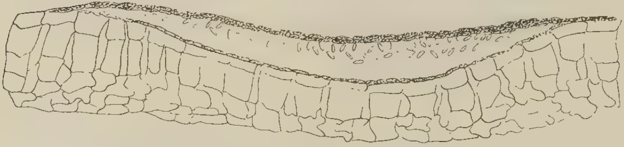


Fig. 69. *Pycnidiopeltis smilacinum* var. *hymenaeae* BAT. & COSTA n. var. Picnostroma em córte longitudinal.

Pycnothyriella BAT.
Typus: *P. discreta* BAT.
in Bol. S.A.I.C. Pern. XIX: 110, 1952.

Mycelium superficiale, planum, brunneum, laminatum, vittaeforme vel irregulare, prosenchymaticum, ex hyphis anastomosantibus, nec liberis, radiatis, setosis, non hyphopodiatis, compositum. Pycnostromata a lamina mycelica tecta, pycnosice evoluta, pseudoostiolata, planoscutata, contigua vel isolata, membranosa. Hymenium inversum. Hypostromata ex hyphis hyalinis penetrantibus usque mesophillum, composita. Conidiophoris filiformibus. Conidia bacillaria, continua, hyalina.

Pycnothyriella discreta BAT. emend.
(loc. cit.).

Mycelium superficiale, planum, brunneum, laminatum, vittaeforme vel irregulare, 1.5—3 mm longum, membranosum, prosenchymaticum, ex hyphis anastomosantibus, non liberis, radiatis, ex cellulis rectangularibus, 5—12.5 × 2.5—4 μ, marginibus integris, ondulatis, non hyphopodiatis, compositum. Setae myceliales atro-

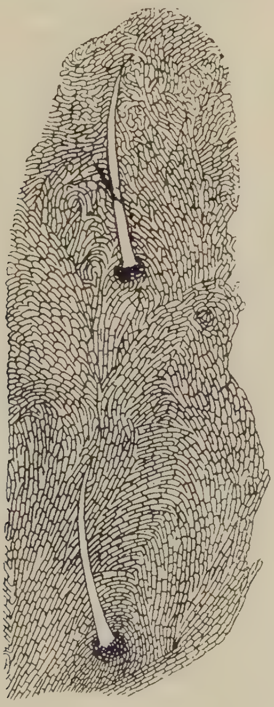


Fig. 70. *Pycnothyriella discreta* BAT. emend.
Micélio laminar, setoso.

brunneae, continuae, acuminatae, parvae, incurvatae, 81.5—165 × 3.5—5 μ. Pycnostromata a lamina mycelica tecta, pycnosice oriunda, plano-scutata, 47.5—75 μ diam., 11.5—15.5 μ alt., pseudo-ostiolata, 10—12.5 μ diam., isolata vel coalescentia. Hypostromata ex hyphis hyalinis, penetrantibus usque mesophyllum, composita.

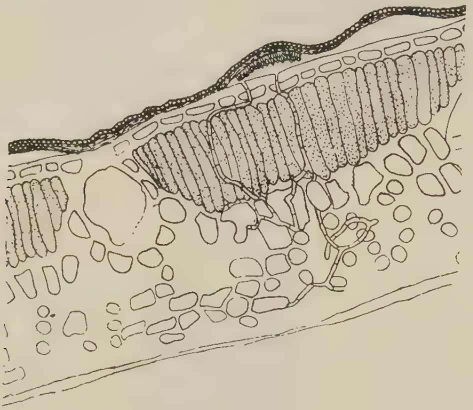


Fig. 71. *Pycnothyriella discreta* Bat. emend.
Córte longitudinal de picnostroma, observando-se o micélio interno.

Hymenium inversum. Conidiophoris filiformibus, hyalinis, 2—5 × 0.5—1 μ. Conidia bacillaria, acrogena, continua, hyalina, 3.5—5 × 1—1.5 μ.

In foliis *Myrciae* sp. socia *Tripospermi* sp. Jardim Zoo-botânico, Dois Irmãos, 24.3.51. Typus, I.P.A., 2478, part. typus 2056, Instituto de Micologia, Universidade do Recife, Prov. Pernambuco, Brasil, Amer. Austr.

Stellopeltis Bat. & Vital, n. gen.

Tipo: *S. philodendricola* (Bat.) Bat. & Vital n. comb.

Micélio superficial, plano, laminar, radiado, de hifas anastomosadas, prosenquimáticas, nunca livres, uniestratoso, de disposição estelar ou estelar-irregular, margens inteiras, porém dentadas, marron, glabras. Picnostromas submicelianos, formados por picnose, marron-negros, sempre salientes, subglobosos pseudo-ostiolados, com himênio invertido; conidióforos filiformes; picnidiosporos cilindráceos, contínuos, hialinos.

Mycelium superficiale, planum, laminatum, unistratosum, radiatum, ex hyphis anastomosantibus, prosenchymaticis, non liberis, stellatum vel irregulariter stellatum, marginibus integris, sed dentatis, brunneis, glabris, non setosis, non hyphopodiatis, compositum. Pycnostromata a lamina mycelica tecta, pycnosice oriunda, atro-brunnea, salientia, subglobosa, pseudo-ostiolata. Hymenium inversum. Conidiophoris filiformibus. Pycnidiosporae cylindraceae, continuae, hyalinae.

Obs.: Difere de **Trichopeltulum** Speg. pela fórma estelar da lâmina miceliana com os bordos e a extremidade das expansões laminares dentados, e picnostromas salientes.

Stellopeltis philodendricola (Bat.) Bat. & Vital n. comb.
Sin.: *Pycnothyrium philodendricola* Bat. *apud* Bat. *et al.*
in An. IV Congr. Nac. Soc. Bot. Brasil, pag. 140, 1953.

Micélio epífilo, superficial, plano, laminar, uniestratoso, mais espesso nas áreas picnostromáticas, de fórma estelar ou estelar-irregular, 4—7 mm de diam., margens inteiras porém dentadas, com numerosos dentes, radiado, 225—340 μ de diam. na área mais larga das expansões laminares, que variam de 65—3050 × 12.5—22.5 μ, também radiadas, terminam indivisas e afiladas ou em 2—4 ramificações divergentes, dentadas; hifas anastomosadas, prosenquimáticas, radiantes e paralelas, constituidas por células retangulares de 2.5—12.5 × 1.5—3.5 μ, glabras, sem hifopódios. Picnostromas desenvolvidos sob a lâmina miceliana, numerosos, convexos,

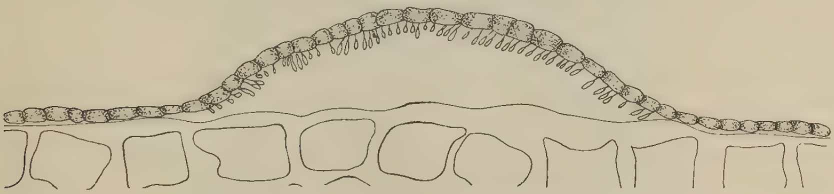


Fig. 72. *Stellopeltis philodendricola* (Bat.) Bat. & Vital.
Córte longitudinal de picnostroma. Orig.

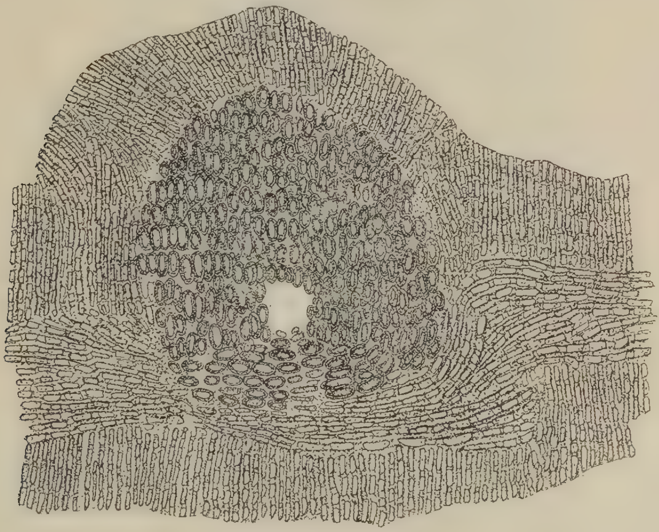


Fig. 73. *Stellopeltis philodendricola* Batista & Vital n. sp.
Detalhe da membrana miceliana a revestir picnostroma. Orig.

salientes, isolados ou contíguos, orbiculares, 65—80 μ de diam., 25—35 μ alt., marron-negros, membranosos, com pseudo-ostíolo de 4.5—9 μ de diam.; parede superior de 7.5—10 μ de espessura; parede inferior indistinta. Himênio invertido. Conidióforos filiformes, 1—3 × 1 μ, hialinos. Picnidiosporos cilindráceos, contínuos, hialinos, 5.5—7 × 1.5—2 μ.

Sôbre folhas de *Philodendron imbe* Schott., associado a *Actinopeltis philodendri* Bat. e *Trispospermum gardneri* (Berk.) Speg. ex Hendrickx. Dois Irmãos, Recife. Tipo I.P.A. 3902, parte do tipo, 43, Instituto de Micologia da Universidade do Recife.

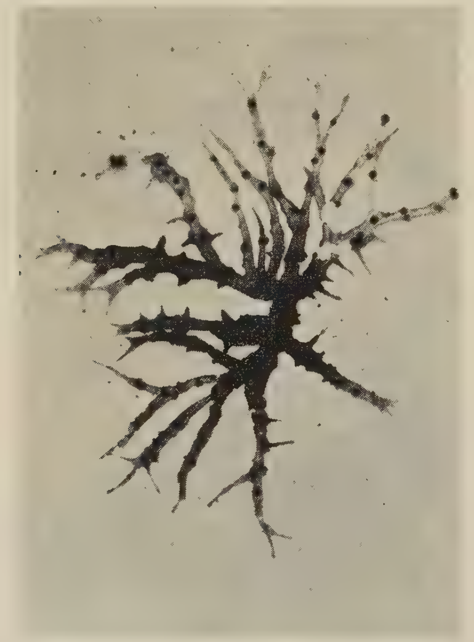


Fig. 74. *Stellopeltis philodendricola* (Bat.) Bat. & Vital
Micélio estelar de expansões dentadas, com picnostromas destacados, marron-negros. Orig.

Mycelium epiphyllum, superficiale, planum, laminatum, stellatum vel irregulariter stellatum, 4—7 mm diam., marginibus integris sed numerose dentatis, radiatis, expansionibus etiam radiatis, 65—3050 × 12.5—22.5 μ, finem acuminatam habentibus, indivisis, vel 2—4 divergentibus ramulis, 225—340 μ diam. in long. diam.; ex hyphis anastomosantibus, radiantibus et parallelis, nec liberis, ex cellulis rectangularibus, 2.5—12.5 × 1.5—3.5 μ, non setosis, sine hyphopodiis, compositum. Pycnostromata a lamina mycelica tecta, pycnosice oriunda, convexa, salientia, orbiculata, 65—80 μ diam., 25—35 μ alt., isolata vel contigua pseudo- ostiolata 4.5—9 μ diam.; pariete superiore 7.5—10 μ cr.; pariete inferiore indistincto.

Hymenium inversum. Conidiophoris filiformibus, continuis, hyalinis, 1—3 × 1 μ. Pycnidiosporae cylindraceae, continuae, hyalinae, 5.5—7 × 1.5—2 μ. — In foliis *Philodendri imbe* SCHOTT. socia *Actinopeltis philodendri* BAT. et *Tripospermi gardneri* (BERK.) SPEG. ex HENDRICKX. — Dois Irmãos Recife. Typus, I.P.A., 3902, part 43, Instituto de Micologia, Universidade do Recife, Prov. Pernambuco, Brasil, Amer. Austr.

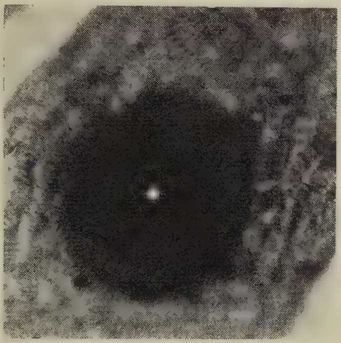


Fig. 75. *Stellopeltis philodendricola* (BAT.) BAT. & VITAL n. sp. Detalhe da estrutura da lâmina miceliana e de picnostroma. Orig.

Stellopeltis philodendricola var. *Compacta* BAT. & VITAL

Micélio epifilo, superficial, laminar até 15 mms de extensão, plano, orbicular-irregular, compacto na area central, com expansões lineares de 100—260 μ de diam, de disposição radiante, margens inteiras com escassos dentes e porção terminal quase sempre dentada, com 1—4 dentes, hifas radiantes e paralelas, anastomosadas, prosenquimáticas, nunca livres, marron. Picnostromas sob o micélio, plano-escutelares, 50—100 μ de diam, 12.5—17 μ de alt, marron-negros, pseudo-ostiolo de 5—7.5 μ de diam, membranosos; parede superior uniestratificada, 2.5—5 μ de espessura; parede basal marron-clara, de cerca de 1 μ de diam; himenio invertido. Conidioforos rudimentares. Picnidiosporos bacilares, continuos, 4.5—7.5 × 1.5 μ, hialinos.

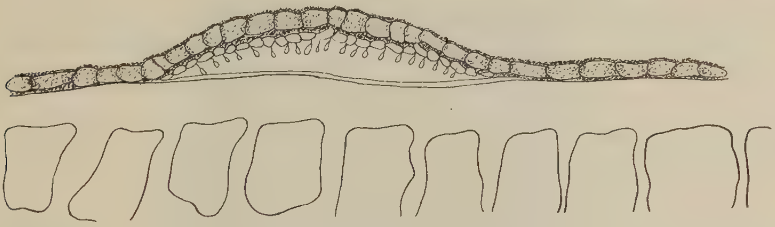


Fig. 76. *Stellopeltis philodendricola* var. *compacta* BATISTA & VITAL. Córte longitudinal de picnostroma.

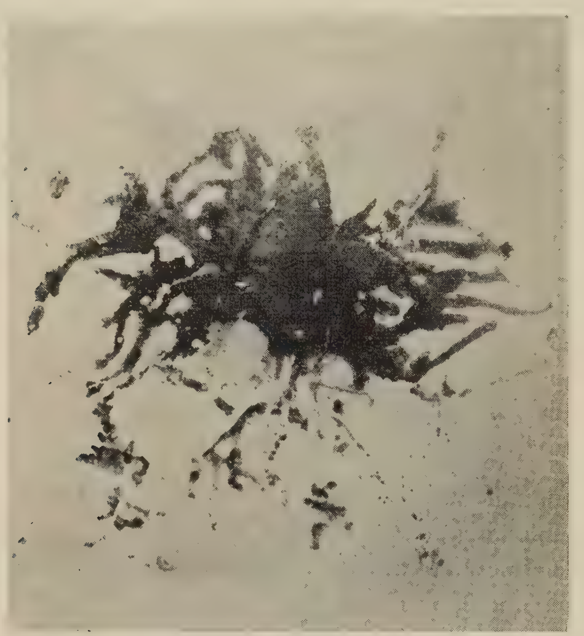


Fig. 77. *Stellopeltis philodendricola* var. *compacta* BAT. & VITAL. Lamina miceliana.

Sôbre folhas vivas de *Eschweilera luschnatii* associado a outros fungos. Milrs. Dois Irmãos. Recife. Leg. J. RODRIGUES, 17.4.53. Tipo, 55, Instituto de Micologia, Universidade do Recife.

A typo differt lamina mycelica compacta in area centrali, usque 15 mm expansa, marginibus raro dentatis et pycnostromate plano-scutato.

Stellopeltis vismiae (BAT. & VITAL) BAT. & VITAL, n. comb.
Sin.: *Pycnothyrium vismiae* BAT. & VITAL apud BATISTA, *in* Bol. Sec. Agr. I. Com. Pern. vol. XVIII: 149, 1951.

Micélio epífilo, superficial, plano, marron, laminar, de aspecto estelar ou estelar-irregular, 2—3 mm de diam., plano, com expansões digitiformes, 190—285 μ de largura na região basal, 78—140 μ de largo na região apical, de extremidades rotundas, margens inteiras, com alguns dentes que chegam a confluir; lâmina uniestratosa, radiada, com hifas anastomosadas, nunca livres, radiadas e paralelas, prosenquimáticas, formadas por células retangulares de 2.5—15 × 2.5—4 μ, glabra, não hifopodiada. Picnostromas desenvolvidos sob a lâmina miceliana, por picnose, convexo-elipsoides, 60—120 μ de diam., 17.5—25 μ de alt., marron-negros, membranosos, com pseudo-ostíolo de 5—7.5 μ de diam.; parede superior de

5—7.5 μ de espessura; parede basal subhialina, 1 μ de diam. Himênio invertido. Conidióforos filiformes, 3—4 × 1 μ, hialinos. Picnidiosporos cilindro-fusoides, contínuos, hialinos, quadrigutulados, 5—7.5 × 1—1.5 μ.

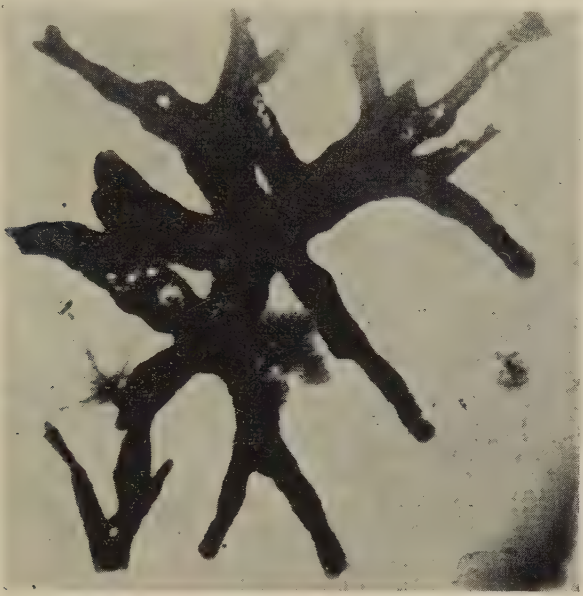


Fig. 78. *Stellopeltis vismiae* Bat. & Vital comb. nov.
Lâmina miceliana com expansões digitiformes, de extremidades rotundas e escassos dentes. Orig.

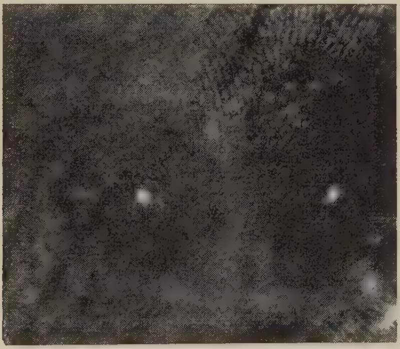


Fig. 79. *Stellopeltis vismiae* Bat. & Vital comb. nov.
Detalhe da estrutura da lâmina miceliana e picnostromas ostiolados. Orig.

Sôbre folhas de *Vismia micrantha* var. *brasiliensis*. Dois Irmãos, Recife. Leg. A. Fernandes Vital, 23.11.51. Tipo 320, I.P.A., parte do tipo 254, Instituto de Micologia, Universidade do Recife.

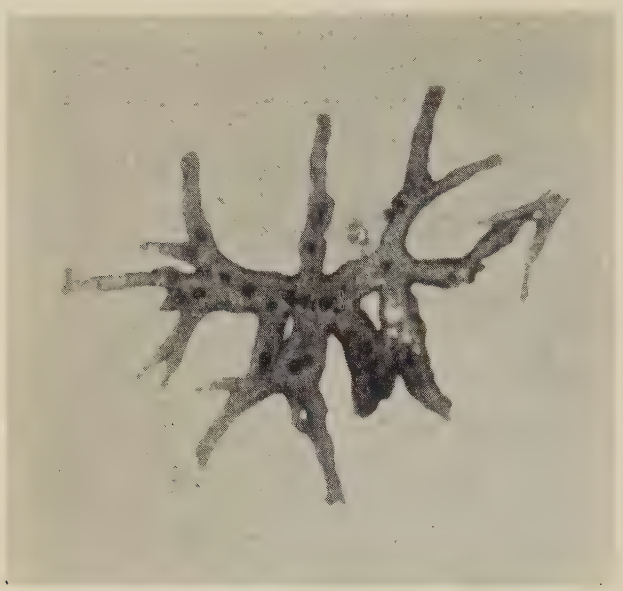


Fig. 80. *Stellopeltis vismiae* BAT. & VITAL comb. nov.
Detalhe da disposição dos picnostromas na lâmina miceliana. Orig.

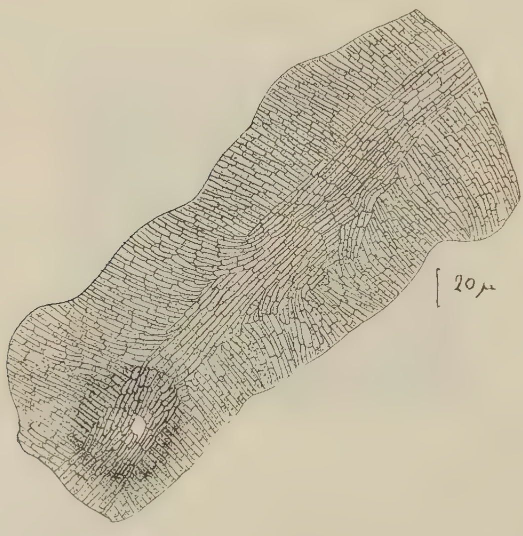


Fig. 81. *Stellopeltis vismiae* (BATISTA & VITAL) BATISTA & VITAL.
Detalhe da estrutura da lámina micélica e picnostroma ostiolado. Orig.

Mycelium epiphyllum, superficiale, planum, brunneum, laminatum, unistratosum, stellatum vel irregulariter stellatum, 2—3 mm diam., expansionibus digitiformibus 190—285 μ basaliter diam., 78—140 μ apicaliter diam., extremitatibus rotundatis, marginibus integris, pauce dentatis, confluentibus; ex hyphis anastomosantibus, radiatis et parallelis, non liberis, prosenchymaticis, ex cellulis rectangularibus, 2.5—15 × 2.5—4 μ, non setosis, non hyphopodiatis, compositum. Pycnostromata a lamina mycelica tecta, pycnosice oriunda, convexo-ellipsoidea, 60—120 μ diam., 17.5—25 μ alt. pseudo-ostiolata 5—7.5 μ diam.; pariete superiore 5—7.5 μ cr.; pariete basali subhyalino, 1 μ cr. efformata. Hymenium inversum. Pycnidiosporae cylindro-fusoideae, 5—9 × 1—1.5 μ, hyalinae, quadriguttulatae. — In foliis *Vismiae micranthae* var. *brasiliensis*. Dois Irmãos, Recife. Leg. A. F. VITAL, 23.11.51. Typus 320, I.P.A., part. 254, Instituto de Micologia, Universidade do Recife, Prov. Pernambuco, Brasil, Amer. Austr.

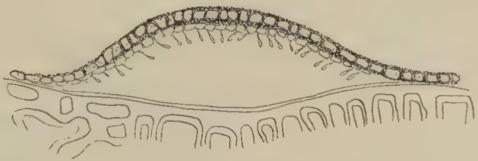


Fig. 82. *Stellopeltis vismiae* (BATISTA & VITAL) BATISTA & VITAL. Córte longitudinal de picnostroma. Orig.

Obs.: A espécie em causa difere de *S. philodendricola* por seu menor tamanho e pela essencial diversidade dos dentes micelianos; os picnidiosporos també́n são quadrigutulados. A constância dos dentes micelianos parece-nos uma boa característica para a separação dêsses dois fungos.

Trichothallaceae BAT. & CIFERRI n. fam.

Tipo: **Trichothallus** STEV.

Fungos de micélio laminar, marron ou subhialinos, rotundos ou alongados, com hifas anostomosadas, nunca livres, radiadas ou paralelas, estéreis, setosos ou não.

Tais fungos representam as fórmas estéreis de Trichopeltinaceae e, provavelmente, também de Trichothyriaceae.

A importância dessa familia resume-se na ordenação das formas estéreis dêsses fungos de micélio laminar que forem encontradas para posteriores estudos da evolução de Trichopeltinaceae.

Mycelium laminatum, rotundatum vel elongatum, ex hyphis non liberis, radiatis vel parallelis, setosis vel non, sterilibus, compositum.

TRICHOTHALLACEAE

CHAVE PARA GÊNEROS

A) Sómente lamina micelica. Picnidios e picnidiosporos ausentes; lâmina miceliana formada por hifas radiantes ou paralelas.

B) Setas ausentes.

C) Lâmina micelica arredondada .... **Strongylothallus** BAT. & CIFERRI

CC) Lâmina miceliana alongada como fita .... **Rhodothallus** BAT. & CIFERRI

BB) Setas presentes; lâmina miceliana alongada como fita .... **Trichothallus** STEV. (Bernice P. Bish. Mus. Bul. 19: 85, 1925)

Rhodothallus BAT. & CIFERRI, n. gen.
Tipo: *R. caseariaefolium* BAT. & CIFERRI & COSTA.

Micélio laminar, alongado, simples ou ramificado, marron a subhialino, hifas anastomosadas, nunca livres, estereis.

Est fungus sterilis. Mycelium laminatum, elongatum, simplex vel ramosum, brunneum vel subhyalinum, ex hyphis non liberis, compositum.

Rhodothallus caseariaefolium BAT., CIFERRI & COSTA, nom. aux.

Fungo esteril. Micélio epifilo, superficial, laminar radiado, plano, membranoso, em fórma de fita com expansões digitiformes,

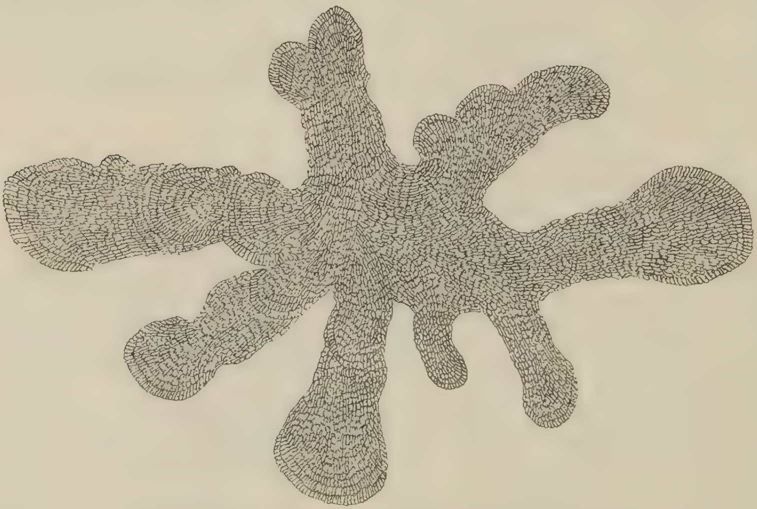


Fig. 83. *Rhodothallus caseariaefolium* BATISTA, CIFERRI & COSTA n. aux. Lámina de micélio, esteril.

bilaterais, de margens inteiras ou lobadas, marron, 425—1000 × 97—190 μ, subhialino a amarelo marron. A estrutura da lamina miceliana é prosenquimática, uniestratosa, integrada por hifas anastomosadas, nunca livres, radiantes, com células cilindricas, de 13.5—22 × 5.5—11 μ. Ausencia de frutificações. Setas e hifopodios também ausentes.

Sôbre folhas vivas de *Casearia* sp. associado a outros fungos. — Mirueira, Recife — Leg. HELES LACERDA, 26.6.55. Tipo, 5777, Instituto de Micologia, Universidade do Recife.

Strongylothallus BAT. & CIFERRI n. gen.

Tipo: *S. scutatus* BAT., CIFERRI & COSTA.

Micelio laminar, rotundo, marron a subhialino, de hifas radiantes, nunca livres, esteril.

Est fungus sterilis. Mycelium laminatum, rotundatum, brunneum vel subhyalinum, ex hyphis radiantibus, non liberis, compositum.

Strongylothallus scutatus BAT., CIFERRI & COSTA n. sp.

Fungo esteril. Colonias micelianas superficiais, marron-claras laminares, plano-escutelares, orbiculares, de margens onduladas, isoladas ou confluentes, 240—470 μ de diam, radiadas, prosenquimáticas, uniestratificadas, constituidas por hifas anastomosadas, nunca livres, tendo celulas cilindraceas de 8—18 × 3—5.5 μ; glabros, nao setosas e sem hifopodios. Himenio ausente.

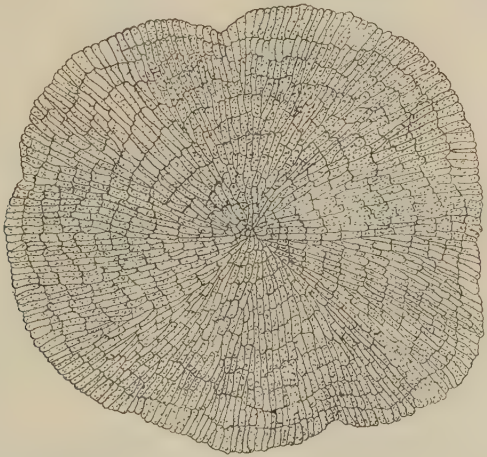


Fig. 84. *Strongylothallus scutatus* BATISTA, CIFERRI & COSTA n. sp. Lámina micélica, esteril. Orig.

Sôbre folhas de *Casearia* sp. associado a *Phragmopeltheca casearicola* BAT. & COSTA — Mirueira, Recife — Leg. HELES LACERDA, 26.6.955. Esp. nº 5775, Instituto de Micología, Universidade do Recife.

Est fungus sterilis; mycelium laminatum, rotundatum, 240—470 μ diam., radians, brunnescens, prosenchymaticum, ex hyphis anastomosantibus, non liberis, compositum. Hymenium nullum.

In foliis *Caseariae* sp. socium *Phragmopelthecae casearicolae* Bat. & Costa — Mirueira, Recife. Leg. Heles Lacerda, 26.6.56. Sp. nº 5775, Instituto de Micologia, Universidade do Recife, Prov. Pernambuco, Brasil, Amer. Austr.

Trichothallus Stev.

Tipo: *T. hawaiiensis* Stev. *in* Bernice P. Bishop Museum, Bul. 19: 85, 1925.

Micélio de Trichopeltinaceae, alongado, setoso. Peritecios ou picnostromas ausentes.

Trichothallus Hawaiiensis Stev.

in Bernice P. Bishop. Mus. Bul. 19: 85, 1925.

Micélio laminar, marron, alongado, irregular, de margens erosadas formado por hifas anstomosadas, paralelas, tendo células retangulares de 10—11 × 4.5—5.5 μ. Setas abundantes, simples, septadas, 90—100 × 7 μ, de apice obtuso ou agudo.

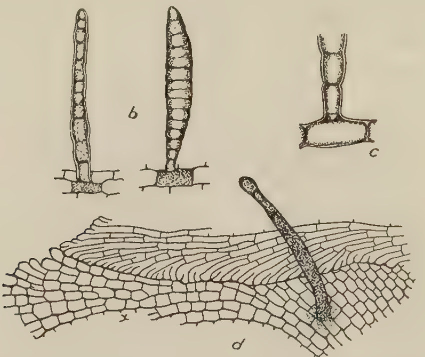


Fig. 85. *Trichothallus hawaiiensis* Stevens.
b) setas dimorficas, da lamina miceliana, sôbre *Scaevola*; c) origem da seta sôbre o micélio laminar; d) laminas distintas de *Trichothallus hawaiiensis* (Y) e *Trichothyrium reptans* (Berk. & Curt) Hughes. Extr. de Hawaiian Fungi, F. L. Stevens, *in* Bernice P. Bishop Mus. Bul. 19 : 85, 1925.

Sôbre *Straussia, Scaevola, Pelea, Metrosideros, Phyllostegia, Rubus, Broussaisis, Alyxia, Vincentia, Freycinetia* — Ilhas do Hawaii.

ÍNDICE DE GÊNEROS E DE ESPÉCIES

Actinothyrium
Actinothyrium smilacis
Allothyriella
A. marcgraviae
A. tecleae
Allothyriopsis
A. landolphiae
Asterinothyriaceae
Asterinothyriaceae — Chave para os gêneros
Asterinothyrium
A. singulatum
Asterostomella
A. acalyphae
A. balanseana
A. capparidis
A. caricae
A. cristata
A. cupaniae
A. dispar
A. erysiphoides
A. fusca
A. horrida
A. indecora
A. kutuensis
A. orthosticha
A. paraguayensis
A. rhaphiostylidis
A. strophanti
A. tonduzi
A. tremae
A. veronicae
A. walleniae
Asterostomopsis
A. ghanaensis
Asterostomula
A. caperoniae
A. loranthi
A. pelladensis
A. pinatubensis
A. premnae
A. pseudospondiadis
A. subreticulata
A subreticulata var. *microspora*
Catenulaster
C. anacardicola
Manginulaceae
Manginulaceae — Chave para os gêneros
Leprieurina winteriana
Palawaniopsis
P. dovyalidis
Parastigmatellina
P. asiatica
Peltasteraceae
Peltasteraceae — Chave para os gêneros
Peltasterella
P. camaragibeana
P. glochidiae
P. lembosicola
P. ocoteae
P. piriensis
P. spissae
Peltasteropsis
P. alaphiae
P. baphiae
P. discretum
P. eugeniae
P. jahnii
P. minutum
P. moquileae
Peltistroma juruanum
Peltistroma pterocelastri
Plectopycnis
P. coccolobae
Plenocatenulis
P. magnoliae
P. osmanthi
Plenotrichaceae
Plenotrichaceae — Chave para os gêneros
Plenotrichella
P. anacardii
P. perseae
Pycnidiopeltis
P. smilacinum
P. smilacinum var. *hymenaeae*

Pycnoseynesia myriadea
Pycnothyriella
  P. discreta
Queenslandia
  Q. pulchella
Rhizothyriaceae
  Rhizothyriaceae — Chave para os gêneros
Rhodothallus
  R. caseariaefolium
Rota
  R. pulchra
Septothyrella
  S. bactridis
  S. nipponica
  S. pernambucensis
Stellopeltis
  S. philodendricola
  S. philodendricola var. *compacta*
  S. vismiae
Stellothyriella
  S. graminis
Strongylothallus
  S. scutatus
Thyriostromella
  T. calophyllii
Trichopeltulaceae
  Trichopeltulaceae — Chave pª os gêneros
Trichothallaceae
  Trichothallaceae — Chave pª os gêneros
Trichothallus
  T. hawaiiensis

ÍNDICE DE SINÔNIMOS

Asterina erysiphoides (*Asterostomella erysiphoides*)
Asteronia (**Asterostomella**)
Asterostomella africana (*Asterostomella paraguayensis*)
  A. banisteriae (*Asterostomella paraguayensis*)
  A. caperoniae (*Asterostomula caperoniae*)
  A. concinna (*Asterostomella paraguayensis*)
  A. dorsteniae (*Asterostomella paraguayensis*)
  A. eugenicola (*Asterostomella strophanti*)
  A. forsteroniae (*Asterostomella tonduzi*)
  A. gregariella (*Asterostomella balanseana*)
  A. hamatula (*Asterostomella walleniae*)
  A. isothea (*Asterostomella paraguayensis*)
  A. lismorensis (*Asterostomella walleniae*)
  A. meliosmaticola (*Asterostomella tonduzi*)
  A. minuta (*Asterostomella tremae*)
  A. ornata (*Asterostomella tremae*)
  A. pelladensis (*Asterostomula pelladensis*)
  A. peraffinis (*Asterostomella walleniae*)
  A. subreticulata (*Asterostomula subreticulata*)
  A. veronicae (*Asteroma veronica*)
  A. visci (*Asterostomella strophanti*)
 sterula? goyazensis (*Leprieurina winteriana*)

Hyphaster (**Asterostomella**)
  H. kutuensis (*Asterostomella kutuensis*)

Leprieurina goyazensis (*Leprieurina winteriana*)
Leptothyriella calophyllii (*Thyriostromella calophyllii*)
  L. graminis (*Stellothyriella graminis*)

Pycnothyrium myriadeum (*Pycnoseynesia myriadea*)
  P. philodendricola (*Stellopeltis philodendricola*)
  P. vismiae (*Stellopeltis vismiae*)

ÍNDICE DE HOSPEDEIROS

- *Alafia scandens*
  - *Peltasteropsis alaphiae*
- *Alsophila rebecca*
  - *Queenslandia pulchella*

- **Alyxis**
  - *Trichothallus hawaiiensis*
- *Anacardium occidentale* L.
  - *Catenulaster anacardicola*
  - *Plenotrichella anacardii*
- *Anona* sp.
  - *Leprieurina winteriana*

- *Bactrys maraja*
  - *Septothyrella bactridis*
  - *S. nipponica*
  - *S. pernambucensis*
- *Bambusa vulgaris*
  - *Pycnoseynesia myriadea*
- *Baphia nitida*
  - *Peltasteropsis baphiae*

- **Broussaisia**
  - *Trichothallus hawaiiensis*

- *Calophyllum floribundum*
  - *Thyriostromella calophyllii*
- *Caperonia palustris*
  - *Asterostomula caperoniae*
- *Capparis cynophallophora*
  - *Peltasteropsis discretum*
  - *Peltasteropsis minutum*
- *Capparis horrida*
  - *Asterostomella horridae*
- *Casearia sp.*
  - *Rhodothallus casearifolii*
  - *Strongylothallus scutatum*
- *Cassipourea gerardi*
  - *Asterostomula pelladensis*
- *Cestrum sp.*
  - *Asterostomella paraguayensis*
  - *Asterostomula subreticulata* var. *microspora*
- *Cestrum pubescens*
  - *Asterostomula subreticulata*
- *Coccoloba paniculata*
  - *Plectopycnis coccolobae*
- **Combretum**
  - *Asterostomella fusca*
- *Costus sp.*
  - *Parastigmatellina asiatica*
- *Cupania revoluta*
  - *Asterostomella cupaniae*

- *Didymopanax morototoni*
  - *Peltasterina lembosicola*
- *Dovyalis rhamnoides*
  - *Palawaniopsis dovyalidis*

- *Eugenia natalitia*
  - *Asterostomella strophanti*
- *Eschweilera luschnatii*
  - *Stellopeltis philodendricola* var. *compacta*

- *Festuca viridula*
  - *Stellothyriella graminis*
- *Fevillea cordifolia*
  - *Asterostomella tremae*
- *Freycinetia sp.*
  - *Trichothallus hawaiiensis*

- *Glochidium ramosii*
  - *Peltasterella glochidiae*
- *Guatteria sp.*
  - *Peltasteropsis jahnii*

- *Helietta cuspidata*
  - *Asterostomella cristata*

Homalium dolichophyllum
  Asterostomella caricae
Hymenaea microphylla
  Pycnidiopeltis smilacinum var. *hymenaeae*

Ilex brunnea
  Asterostomula pinatubensis

Jasminum sp.
  Peltasterella spissae
Jasminum tortuosum
  Asterostomella erysiphoides
Justicia flava
  Asterostomella walleniae

Landolphia owariensis
  Allothyriopsis landolphiae
Lawsonia inermis
  Asterostomopsis ganae

Magnolia glauca
  Plenocatenulis magnoliae
Marcgravia rectiflora
  Allothyriella marcgraviae
Metrosideros sp.
  Trichothallus hawaiiensis
Moquilea tomentosa
  Peltasteropsis moquileae
Muhlenbergia filiformis
  Stellothyriella graminis
Myrciae sp.
  Pycnothyriella discreta

Ocotea sp.
  Peltasterella camaragibeana
  Peltasterella ocoteae
Osmanthus americanus
  Plenocatenulis osmanthusi

Pelea sp.
  Trichothallus hawaiiensis
Persea gratissima
  Plenotrichella perseae
Phleum sp.
  Stellothyriella graminis
Philodendron imbe
  Stellopeltis philodendricola
Phyllostegia
  Trichothallus hawaiinsis
Pleioceras barteri
  Asterostomella walleniae
Prenna sp.
  Asterstomula premnae
Pseudospondias microcarna
  Asterostomula pseudospondiadis
Pterocelastrus galpini
  Peltistroma pterocelastri

Rhaphiostylis beniensis
  Asterostomella rhaphiostylidis
Rollinia silvatica
  Asterostomella fusca
Rubus sp.
  Trichothallus hawaiiensis
Rutaceae
  Asterostomella paraguayensis

Scaveola sp.
  Trichothallus hawaiiensis
Smilax sp.
  Pycnidiopeltis smilacinum
Smilax rotundiofolii
  Actinothyrium smilacis
Solanaceae
  Asterostomella balanseana
Straussia sp.
  Trichothallus hawaiiensis

Teclea nobilis
  Allothyriella tecleae
Trema micrantha
  Asterostomella tremae
Trichocladus ellipticus
  Asterinothyrium singulatum
  Peltasterella piriensis
Triumfetta semitroloba
  Asterostomella paraguayensis

Veronica derwentiae
  Asterostomella veronicae
Vincentia sp.
  Trichothallus hawaiiensis
Viscus obscurum
  Asterostomella strophanti
Vismia micrantha var. *brasiliensis*
  Stellopeltis vismiae

INTORNO AD UN LIEVITO OSMOFILO SISTEMATICAMENTE PROSSIMO A SACCH. ELEGANS LODDER ET KREGER-VAN RIJ

O. Verona & O. Zardetto de Toledo

(*Istituto di Microbiologia Agraria e Tecnica dell'Universita' - Pisa*)

(9.II.1959)

Studiando una serie di lieviti isolati da uva raccolta nei pressi di Campinas nello Stato di S. Paolo in Brasile, due ceppi ci hanno lasciato, dal punto di vista sistematico, un poco perplessi. Si è proceduto, pertanto, ad uno studio di dettaglio, studio che si è portato su quattro isolati monocellulari ottenuti da uno dei ceppi. Indicheremo tali isolati con le lettere A-B-C-Z.

DESCRIZIONE TECNICA

Sviluppo in malto

Dopo 3 giorni a 26° C. cellule subovali od ovali misuranti nell' isolato A $\mu(3.6—4.8) \times (3.6—7.2)$ con presenti cellule anche più lunghe fino a μ 9.6, ma rare; negli altri isolati cellule ovalari di $\mu(3.6—4.8) \times (3.6—7.2)$.

Sedimento, talvolta flocculoso, al fondo; liquido limpido; non velo; possibile formazione di anello non continuo nelle vecchie colture.

Sviluppo in agar-malto

Dopo 3 giorni a 26° C. cellule subovali od ovali di $\mu(3.6—6) \times (3.6—7.2)$ od anche un poco più lunghe nell'isolato A e $\mu(3.6—4.8) \times (4.8—7.2)$ negli altri. Nelle vecchie colture, specie il ceppo A, dà origine a cellule spesso provviste di tubuli. Sviluppo non rapido di vegetazione non molto estesa, pastosa, bianco-giallastra, generalmente liscia.

Colture su lamina

Microcolture su agar-patata non rivelano formazione di vero pseudomicelio, ma solo tenui formazioni arbuscolari.

Sporulazione

Facile ed abbondante anche in agar-malto. Nell'isolato A gli aschi si formano in prevalenza previa copula isogamica. Contengono 1—2 od anche 3 spore globose, più difficilmente ovoidi, di μ 3.2—3.8, con spesso una goccia oleosa. Negli isolati B-C-Z si ha copula

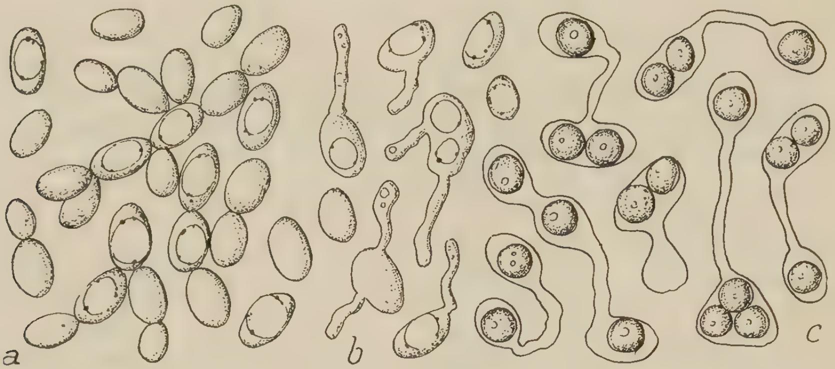


Fig. 1. *a*) giovani cellule in malto; *b*) cellule in vecchie colture in agar-malto; *c*) cellule sporificate.

isogamica come in A, ma anche formazione di aschi non preceduta da copula; in tal caso gli aschi possono contenere anche 4 spore.

Fermentazione

glucosio	+	maltosio	—
saccarosio	+ (o —)	lattosio	—
galattosio	—	raffinosio	—
		inulina	—

La fermentazione del saccarosio merita alcuni chiarimenti.

Gli incerti risultati avuti usando i test standard hanno suggerito di ripetere più volte la prova anche in condizioni di diverso pH. Si è così tamponato il mezzo base a pH 5 e a pH 7 aggiungendo il saccarosio asetticamente al mezzo già sterile. Tanto, per evitare che anche tracce minime di saccarosio venissero invertite durante la sterilizzazione. Incubazione a 26° C., prove in triplo. I risultati avuti hanno consentito di poter stabilire che:

a) a pH 5 la fermentazione del saccarosio è sempre positiva verificandosi entro l'8°—13° giorno, raramente più tardi;

b) a pH 7 la fermentazione del saccarosio, seguita per 30 giorni, può, in qualche caso, risultare negativa. Quando è positiva si svolge tuttavia con grande lentezza, cioè dopo 18—25 giorni dalla semina.

Riassumendo la fermentazione del saccarosio è positiva, se pure talvolta relativamente lenta, in mezzo acido. Può risultare negativa e comunque molto lenta a pH neutro.

Utilizzazione dei composti di C

glucosio	+	maltosio	—
galattosio	— (o poco)	lattosio	—
saccarosio	+ (o —)	raffinosio	—

inoltre:

xilosio	—	ribosio	—
arabinosio	—	inulina	—
sorbosio	+	glicerina	+
cellobiosio	—	mannite	+ (o poco)
ramnosio	—	dulcite	—

Assimilazione dei composti azotati

I nitrati non vengono assimilati. Positiva l'assimilazione di: solfato ammonico, urea, asparagina, peptone.

Utilizzazione dell'etanolo come unica sorgente di C

Sviluppo assente o molto scarso.

Scissione dell'arbutina: negativa.

Formazione di esteri: negativa.

Sviluppo in assenza di vitamine

Nel liquido di Williams senza vitamine lo sviluppo è moderatamente positivo; è migliore se presenti vitamine.

Sviluppo in soluzioni concentrate di zucchero

Positivo anche in mezzi contenenti gr. 80 di zucchero in cc. 100 di acqua di lievito.

Sviluppo a 37° C.: positivo.

Rendimento in alcool

In mosto d'uva l'attività fermentativa è già esaurita (a 25—26°C) all. '8°—10° giorno.

In un mosto contenente il 23.4 % di zucchero i quattro isolati dettero, al 20° giorno:

alcool etilico (in volume)	6.49 — 6.61 %
ac. volatile (in ac. acetico)	0.42—0.48 ‰

DISCUSSIONE

I quattro isolati non si dimostrano fondamentalmente diversi nonostante le dimensioni delle cellule appaiano, nell'isolato A, leggermente maggiori che negli isolati B-C-Z. Tutti gli altri caratteri, di contro, sono risultati uguali. E' vero, d'altronde, che tutti gli isolati provengono da un medesimo ceppo.

Per quanto si riferisce al riferimento sistematico la dimostrata capacità, dei nostri isolati, a svilupparsi in soluzioni molto concentrate di zucchero ci suggerì di procedere, in prima istanza, ad un confronto con le specie qualificate „osmofile'', comunque con le specie fermentanti il solo glucosio 1) o glucosio e saccarosio.

In effetti, dal punto di vista morfologico e sporologico, i nostri isolati richiamano molto da vicino *Sacch. mellis* (Fabian et Quinet) Lodder et Kreger-van Rij, mentre da un punto di vista fisiologico ci è sembrato essere più vicini a *Sacch. elegans* Lodder et Kreger-van Rij, lievito capace di svilupparsi, come noi abbiamo osservato, in presenza di elevate concentrazioni zuccherine e pertanto riferibile al gruppo degli osmofili.

Conseguentemente si è proceduto ad un confronto diretto con queste specie cortesemente forniteci dalla Signora Dr. Kreger-van Rij che qui vivamente ringraziamo.

Invero, nonostante la simiglianza dei caratteri morfologici e sporologici, un ravvicinamento a *Sacch. mellis* non appariva molto giustificato dal momento che, *Sacch. mellis*, fermenta solo il glucosio.

Dovevamo però tener conto che Scarr, nel 1951 2), dava come possibile nei lieviti osmofili fermentanti il solo glucosio, una graduale inversione del saccarosio e questo a seguito di formazione di acidi.

Più giustificato appariva invece un accostamento a *Sacch. elegans* dato che questo fermenta sempre, sia pure molto lentamente, il saccarosio.

Il confronto diretto dei nostri isolati con *Sacch. mellis* e *Sacch. elegans* portò ai risultati che si trovano trascritti in Tabella e che indicano, come accennato, i molti punti di contatto con questa ultima specie.

Rispetto al tipo si notano comunque piccole differenze nei riguardi morfometrici. Quindi: *a*) nella fermentazione del saccarosio che nei nostri isolati può essere non lenta a pH 5 e molto lenta od anche negativa a pH 7; *b*) nella assimilazione del galattosio che *Sacch. elegans* assimila, se pure assai poco; *c*) nella possibilità, da parte dei nostri isolati, di utilizzare il sorbosio.

---

1) Nel gruppo dei lieviti fermentanti il solo glucosio si trovano, così distinte, le seguenti specie:

cellule misuranti	sviluppo lento	*S. mellis*
μ(2—5) × (3—7)	sviluppo normale	*S. bisporus*
cellule misuranti	coltura liscia	*S. acidifaciens*
μ(3—7) × (5—15)	coltura increspata	*S. baili* *S. scandinavicus* *S. tellustris*

Noi pensiamo che sarebbe interessante un più esteso esame comparativo di queste specie e ciò allo scopo di trovare elementi di maggiore differenziazione. Infatti, in base a questi soli caratteri — fermi ed uguali rimanendo tutti gli altri — in ordine a quanto oggi è acquisito in materia di variazioni si può rimanere perplessi nell'accettare come valide specie che tra loro differiscono o differirebbero solo per uno sviluppo più o meno veloce o l'aspetto liscio o rugoso delle vegetazioni in agar. Avendo tutte lo stesso abito morfologico, sporologico, zimologico ed auxologico, sarebbe forse biologicamente più esatto passarle a varietà di una unica specie. Sempre che, come si è detto, non sussistano altri caratteri differenziali.

2) Scarr, M. P.: J. gen. Microbiol., **5**, 704—713, 1951.

TABELLA

	forma e dimensioni (agar-malto) 3° g. a 26° C.	Fermentaz.ne del saccarosio	fermentaz.ne del raffinosio	Assimilazione composti del carbonio						urea, asparagina peptone, NH_4	sviluppo a 37° C.	sviluppo in soluzioni zuccherine concentrate
				galattosio	saccarosio	sorbosio	arabinosio xilosio ramnosio ribosio cellobiosio	glicerina mannite	etanolo			
S. mellis	rotonde di μ (2.6—5.2) × (3.4—6.2) e ovali di μ(2.6—4.5) × (3.5—10.5)	—	—	+ o —	—	—	—	+	—	+	+	+
S. elegans	ovali di μ(3.5—6) × (4.5—9)	+ ma molto lenta sia a pH 5 che a pH 7	+ (poco)	+ (poco)	+ (lento)	— o molto poco	—	+	— o poco	+	+	+
isolato A	subovali od ovali di μ(3.6—6) × (3.6—7.2) con rare cell. più lunghe	+ a pH 5 + o — sempre molto lenta a pH 7	—	— o molto poco	+ (o —)	+	—	+	— o poco	+	+	+
isolati B-C-Z	subovali di μ(3.6—4.8) × (3.6—7.2)	id.	—	— o molto poco	+ (o —)	+	—	+	— o poco	+	+	+

Almeno pro tempore crediamo di poter interpretare i nostri isolati come una varietà di *Sacch. elegans* Lodder et Kreger-van Rij; Indichiamo questa varietà come:

Sacch. elegans Lodder et Kreger-van Rij, var. *intermedia*, n. var. (pro tempore).

Riassunto

Viene descritto un lievito osmofilo, isolato da mosto d'uva naturale, tassonomicamente vicino a *Sacch. elegans* Lodder et Kreger-van Rij.

Questo lievito viene riguardato come *Sacch. elegans* var. *intermedia* nov. var. (pro tempore).

Summary

Here is described an osmophilic yeast isolated from natural grape must which is taxonomically similar to *Sacch. elegans* Lodder et Kreger-van Rij.

This yeast is regarded as *Sacch. elegans* var. *intermedia* nov. var. (pro tempore).

Resumo

Nêste trabalho descrive-se uma levedura osmofila isolada de môsto de uva natural taxonomicamente proxima a *Sacch. elegans* Lodder et Kreger-van Rij.

Esta levedura è considerada então, como *Sacch. elegans*, var. *intermedia* nov. var. (pro tempore).

GROWTH AND NUTRITION OF PSEUDOARACHNIOTUS ROSEUS IN SUBMERGED CULTURE

Harold H. Kuehn & Paul F. Crosby
(*Grain Processing Corporation, Muscatine, Iowa*).

(24.III.1959)

Reports in the literature concerned with physiological investigations of representatives of the Gymnoascaceae are limited in number. Furthermore, with one or two exceptions there have been no studies of the metabolic products formed by species of this group of fungi. With the advent of submerged culture growth techniques for microorganisms, and the rapid production of material made possible by its application (Kluyver & Perquin, 1933), it appeared desirable to undertake an examination of the Gymnoascaceae in order to obtain specific information. In preliminary experiments directed toward the feasibility of studying the Gymnoascaceae in submerged culture, certain aspects of the behavior of *Pseudoarachniotus roseus* Kuehn, especially the rapidity of its growth, prompted its selection as the organism for our initial studies.

The work on the Gymnoascaceae has been limited almost entirely to *Gymnoascus setosus* Eidam and *Byssochlamys fulva* Olliver & Smith. Hawker (1936) cultured *G. setosus* on an agar medium which contained 0.5 % glucose, 0.35 % KNO_3, 0.075 % $MgSO_4$ and 0.175 % KH_2PO_4 to which addition of either a crude lentil extract or an inositol-free fraction of the crude extract failed to improve the fruiting of the fungus. Schopfer & Blumer (1940) reported that *G. setosus* grew on a mineral-dextrose medium which contained asparagine. The addition of thiamin or its intermediates had no effect on the growth of the fungus. Robbins & Kavanagh (1942) reviewed this work with *G. setosus*. More recently Nyuksha (1953, 1955, 1957) studied *G. setosus* more thoroughly and reported that pigmentation and growth occurred normally on sugars (only feebly on lactose), starch and cellulose but not on citric acid. The best source of nitrogen was peptone while in descending order the mold also used gelatin, KNO_3, asparagine and $(NH_4)_2SO_4$. He also reported that the fungus could grow well on a medium which lacked a nitrogen source, but on such a medium no pigment was elaborated.

Raistrick & Smith (1933) reported that *Byssochlamys fulva* converted glucose to mannitol. Pectin polygalacturonase was found in *B. fulva* by Holden (1950) and also by Reid (1950, 1952). Reid also found pectin esterase, arabanase and galactanase to be present, while HULL (1939) and BEAVEN & BROWN (1949) found protopectinase in this microorganism.

BERTHELOT (1899) reported that an unidentified species of *Gymnoascus* fixed atmospheric nitrogen. However, HEINZE (1906) stated that the results were caused by impure cultures.

KAROW & FOSTER (1944) found that an unidentified species of *Gymnoascus* produced clavacin when it was incubated for 7—10 days in Czapek-Dox medium supplemented with 3 % corn steep liquor. The authors referred to this culture as — 5070.1 of the Thom culture collection. KUEHN subsequently learned that this strain is also designated as NRRL 1678 and identified this culture to be *Byssochlamys nivea* (1957a).

MATERIALS AND METHODS

The microorganism used in this study was *Pseudoarachniotus roseus* KUEHN, NRRL 1677, which is also the type culture. This strain was isolated from an ulcerated gizzard of a chicken, in the vicinity of Tuscon, Arizona, sometime prior to 1934. Dr. THOM identified it as a *Gymnoascus* sp., and retained it in his collection as 5439.

In preparation for a detailed study of the biosynthetic capacities of *P. roseus* it became necessary to develop a nutrient medium in which optimum growth could be produced in a relatively short period of time. Information regarding the application of submerged culture technique to the growth of this organism, or any other of the Gymnoascaceae, is lacking completely. However, since cultures of *P. roseus* and other Gymnoascaceae grew well on YpSs agar as well as on Sabouraud's agar (KUEHN, 1955a, 1955b, 1956, 1957a 1957b) the constituents of these media were used in preliminary experiments.

Sabouraud's broth was used with the following constitution: 1 % peptone, 4 % dextrose and pH 7.2. The YpSs medium was as follows: 1.5 % soluble starch, 0.4 % yeast extract, 0.1 % K_2HPO_4 and 0.05 % $MgSO_4 \cdot 7H_2O$. The pH was 7.2 prior to autoclaving and 7.4 afterward. Since the YpSs medium permitted more rapid growth than Sabouraud's broth, various modifications of the former medium were used for further experimentations. Various carbon compounds were substituted for soluble starch to determine the best carbon sources for growth of *P. roseus*, also, various nitrogen-containing compounds were substituted for yeast extract in this medium to determine which nitrogenous compounds were most suitable for growth. The single carbohydrates were routinely employed in concentrations of 1.5 % while the potential nitrogen sources were incorporated at levels of 0.4 %. The amount of available carbon present in some of the organic nitrogen sources was not taken into account in the carbohydrate concentrations.

Cultures were grown in 500 ml Erlenmeyer flasks containing 100 ml of nutrient medium. The pH of the medium was adjusted with a Beckman Zeromatic pH meter prior to autoclaving and the pH was checked again following sterilization. Flasks were capped with gauze-covered cotton pads which were held in place with inverted paper drinking-cups, whose bottoms were removed. The media were autoclaved at 15 pounds pressure for 30 minutes at 251° F. After inoculation the flasks were placed on reciprocating shaking machines making 93 complete 4½ inch strokes per minute.

It was found in preliminary experiments that a reciprocating shaker permitted more growth than did a rotary shaker. In the former the growth was of a fine, feathery type, while in the latter the mycelium was in the form of small, round pellets. Evidently fragmentation of the mycelium proceeded better on the reciprocating shaker thus allowing more dispersed growth and hence better utilization of the available nutrients. Thus, the reciprocating type of shaker was used for all experiments.

Typical growth consisted of minute, feathery mycelial strands which varied in color from white to orange-yellow depending upon the constituents of the medium and upon the length of incubation. Occasionally the growth occurred as small pellets of mycelium even on the reciprocating shaker, but this condition usually was associated with suboptimal media or other suboptimal conditions for growth.

Following the desired incubation time the contents of each flask were centrifuged at 2100 rpm in 250 ml centrifuge bottles for 8 minutes in order to remove the cellular material. The pH of the supernatant was recorded. The centrifugate was washed with distilled water and recentrifuged in 40 ml centrifuge tubes at 2500 rpm for 8 minutes in order to remove any residual, undissolved constituents of the medium which might have contributed toward an erroneous mycelial weight. The centrifuged cell material was then washed quantitatively into tared aluminium weighing pans and the dry mycelial weights were determined after drying overnight in a constant-temperature oven at 100° C. Each value reported in this study is the average of two flasks, and in certain instances experiments were repeated in order to verify the results.

During the preliminary experimentation flasks were inoculated with spores transferred from stock slants. However, it was found that a more uniform inoculum could be obtained by use of 2 % of a 3 day old submerged culture of YpSs medium.

RESULTS AND DISCUSSION

A. Relation of the length of incubation to growth.

A study was made to determine the incubation time required to permit maximum growth in the YpSs medium. A number of replicate. flasks, pH 6.0, were placed on the shaker simultaneously at 28° C. At intervals during the incubation period duplicate flasks were removed from the shaker, the pH was determined, and the growth was harvested in the manner previously described. The results, shown in Figure 1, indicated that maximum or near-maximum growth was attained in about 88 hours, and thereafter the weight of mycelium remained almost constant with a slight decrease with the inception of autolysis. Based upon this information, experiments incubated at 28° C were terminated in about 3½ days during which time the pH gradually increased to 7.5—8.0.

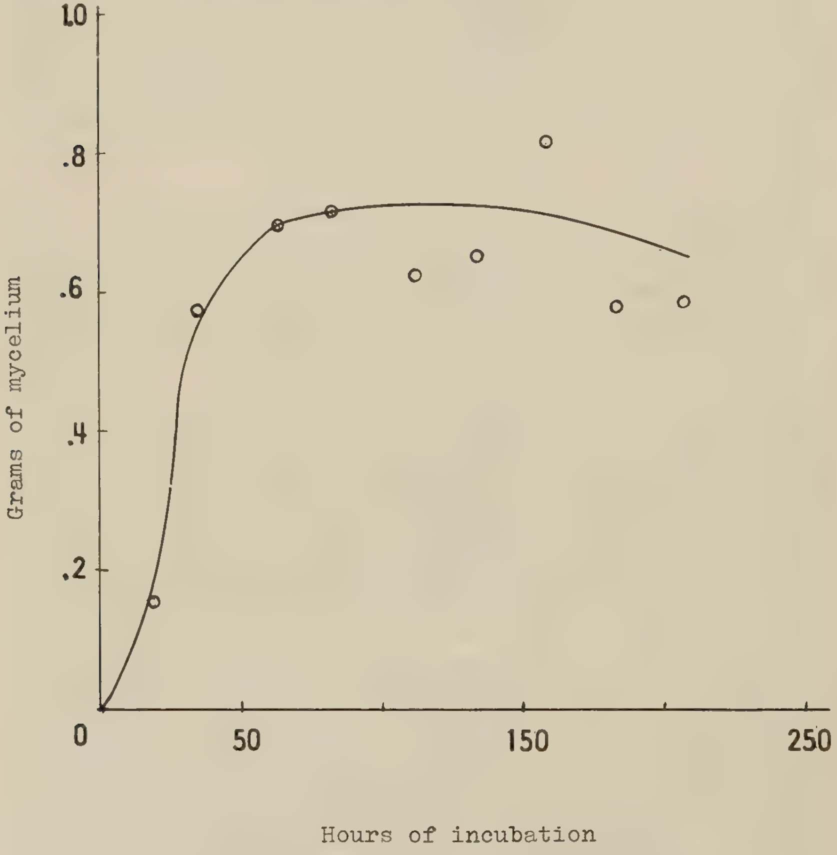


Fig. 1. Mycelial weight of *P. roseus* versus fermentation time in hours.

B. The effect of initial pH of the medium.

The non-adjusted pH of the YpSs agar medium as well as of the broth was 7.2. However, there was no basis for considering this to be the optimum initial pH for the growth of *P. roseus*. Therefore, the initial pH of the YpSs broth in a series of flasks was adjusted to various levels prior to autoclaving. It was found that sterilization did not significantly affect the initial pH. Incubation was carried out at 28° C for 84 hours. The results presented in Table I indicate that in the pH range of 6.0—9.0 the initial pH was not critical.

TABLE I.

Effect of initial pH of the medium upon the growth of P. roseus.

Flask	Initial pH	Final pH	Mycelial weight g/100 ml
1	3.0	4.3	0.049
2	,,	4.2	
3	4.0	5.1	0.268
4	,,	4.9	
5	5.0	5.2	0.517
6	,,	5.6	
7	6.0	6.7	0.553
8	,,	6.6	
9	7.2	7.2	0.557
10	,,	7.3	
11	8.0	7.5	0.529
12	,,	7.5	
13	9.0	7.5	0.565
14	,,	7.5	

In all tables, the final pH is recorded for individual flasks, while the mycelial weight represents the average of the replicate flasks.

C. The utilization of different carbon compounds.

In a preliminary experiment urea supported significantly more growth than yeast extract in the YpSs medium. Therefore, urea was used as the nitrogen source in the following carbon utilization studies.

Pseudoarachniotus roseus previously had been cultured only upon four sources of carbon: sucrose, maltose, glucose and soluble starch. In this study a total of 30 carbon sources was added singly at a level of 1.5 % to individual flasks which contained the following medium: 0.4 % urea, 0.1 % K_2HPO_4 and 0.05 % $MgSO_4.7H_2O$. The pH was adjusted to 6.0 following addition of the carbon compound. Table II presents the results of this experiment which was incubated for 5 days at 28° C. The compounds are listed in descending order of availability.

Although three simple, defined carbon sources supported better growth than soluble starch, the latter compound was used in succeeding experiments. The reason for this was the slight carmelization of maltose and xylose during autoclaving. Since this did not occur with soluble starch its use obviated the necessity of separate sterilization of the carbon source and subsequent aseptic addition to the test flasks. Also, maltose itself lent a slight yellow-brown color to the medium which interfered with investigations on the pigments produced in the various experiments.

TABLE II.

Availability of various carbon compounds for growth of P. roseus.

Compound	Final pH	Mycelial weight g/100 ml
maltose	8.9	0.711
corn starch	8.2	0.697
dextrin	9.0	0.613
xylose	8.9	0.608
soluble starch	8.9	0.600
cellobiose	8.3	0.596
fructose	8.9	0.582
mannose	8.9	0.532
glucose	8.9	0.490
sodium acetate	9.4	0.321
lactic acid	8.3	0.283
inositol	8.9	0.168
calcium gluconate	8.0	0.154
propionic acid	8.0	0.137
sucrose	8.9	0.120
rhamnose	8.2	0.110
sorbose	8.6	0.100
raffinose	8.3	0.090
itaconic acid	8.0	0.086
sorbitol	8.3	0.084
gluconic acid	8.2	0.077
succinic acid	8.1	0.073
lactose	7.8	0.069
adipic acid	8.0	0.056
malic acid	8.2	0.052
sodium citrate	8.2	0.051
oxalic acid	8.1	0.048
citric acid	7.8	0.041
kojic acid	7.1	0.038
gallic acid	6.5	0.000

D. The utilization of different nitrogen compounds.

Pseudoarachniotus roseus was maintained on stock slants of either YpSs or Sabouraud's agar, which contain as the nitrogen source yeast extract and peptone respectively. This organism also has been cultured on Czapek's agar with $NaNO_3$ as the nitrogen source, but on this medium growth was very limited and sparse. Forty-three different nitrogen-containing compounds were tested as sole nitrogen sources in concentrations of 0.4 % by substituting each of them for yeast extract in the YpSs medium. All flasks were adjusted to pH 6.0 and were incubated at 28° C for 84 hours. The results are presented in Table III with the compounds listed in descending order of availability. Two sets of controls were run, one with yeast extract and the other with peptone.

It is well known that microorganisms utilize various compounds at different rates so that while growth with different compounds

TABLE III.

Growth of P. roseus on different nitrogen sources.

Compound	Final pH	Mycelial weight g/100 ml
ammonium acetate	7.2, 7.7	1.058
bacto asparagine	6.5, 6.3	1.041
peptone	7.6, 7.5	0.925
l-glycine	7.9, 8.5	0.884
yeast extract	7.2, 7.2	0.822
urea nitrate	8.2, 8.0	0.758
urea	8.9, 8.9	0.728
sodium asparaginate	7.6, 7.8	0.684
glutamic acid	7.7, 7.8	0.670
l-asparagine	7.0, 6.7	0.605
sodium nitrate	8.2, 8.2	0.597
l-arginine HCl	5.9, 5.7	0.593
beta alanine	5.5, 5.8	0.575
dl-aspartic acid	7.5, 7.0	0.554
calcium nitrate	7.3, 7.6	0.533
dl-asparagine	6.3, 6.1	0.533
ammonium citrate	4.6, 4.6	0.513
ammonium carbonate	4.1, 4.0	0.505
urea oxalate	8.5, 8.6	0.500
ammonium bicarbonate	4.0, 4.0	0.498
ammonium lactate	8.5, 4.7	0.480
dl-isoleucine	5.3, 5.4	0.471
ammonium tartrate	4.3, 4.3	0.448
l-tyrosine	6.0, 5.9	0.444
l-cystine	5.9, 6.0	0.438
25 % choline chloride	6.4, 6.2	0.434
$NH_4H_2PO_4$	4.0, 4.1	0.409
sodium nitrite	6.8, 7.2	0.398
dl-histidine HCl	5.5, 5.7	0.363
potassium nitrate	8.3, 8.5	0.358
l-valine	6.0, 6.0	0.347
corn steep liquor	6.2, 6.2	0.346
dl-serine	4.9, 4.8	0.343
ammonium chloride	3.9, 3.9	0.341
ammonium sulfate	3.9, 3.9	0.339
dl-methionine	4.8, 4.7	0.338
vitamin-free casein hydrolysate	5.2, 5.0	0.335
l-tryptophan	5.9, 5.9	0.312
ammonium molybdate	5.6, 5.6	0.280
choline citrate	6.0, 6.0	0.265
ammonium nitrate	4.5, 4.1	0.177
$(NH_4)_2HPO_4$	4.0, 4.0	0.170
l-lysine	4.2, 4.4	0.107

in a unit time might be quite dissimilar the final total growth might be comparable. Hence, for definitive studies on the availability of compounds it is necessary to obtain a growth curve with relation to time for each test compound. In this study we were concerned primarily with a determination of those compounds which would permit maximum growth in the least time and therefore the mycelium was harvested at only one sampling time.

It can be seen from Table III that ammonium acetate and bacto asparagine were the two best sources of nitrogen. Both were superior to either peptone or yeast extract.

The various nitrogen sources listed in Table III were added at a concentration of 0.4 %, a value which was selected since in the YpSs medium the yeast extract was present at that level. Varying levels of ammonium acetate were investigated to determine the optimum level for growth. The test medium consisted of 1.5 % soluble starch, 0.1 % K_2HPO_4 and 0.05 % $MgSO_4.7H_2O$ adjusted to pH 6.0.

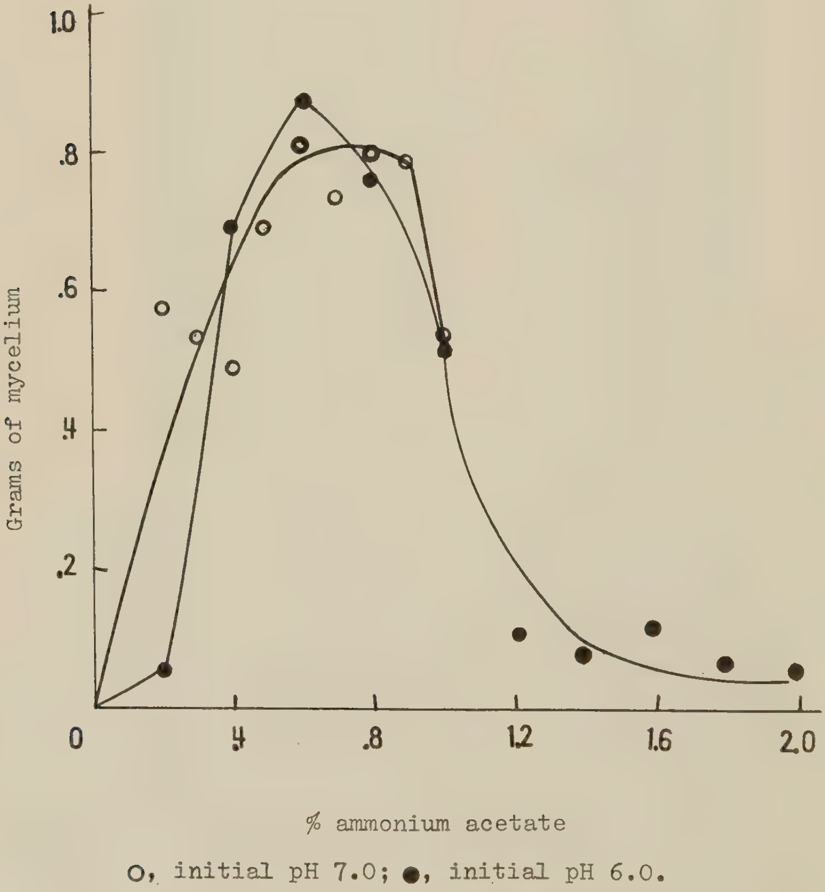


Fig. 2. Growth response of *P. roseus* to various concentrations of ammonium acetate at two different pH levels.

It was decided to check availability of various concentrations of ammonium acetate at more than one pH level. Figure 2 illustrates the response of *P. roseus* to various levels of ammonium acetate at pH 6.0 and at 7.0 during a 4 day incubation period. A 0.6 % concentration of ammonium acetate was found to be optimum at either pH level. A similar experiment was conducted using bacto asparagine at pH 6.0, since this compound proved to be the second most rapidly assimilated nitrogen source (see Table III). Again a 0.6 % concentration of the nitrogen source proved to be most favorable for mycelial production.

The initial experiment to determine the optimum pH level for this microorganism (see Table I) was conducted using yeast extract as the nitrogen source. A redetermination of the best level of acidity was made using ammonium acetate as the nitrogen source since the availability of ammonium acetate was shown to be affected by pH (see Figure 2). The results showed that the optimum initial pH was between 7.0 and 8.0 and therefore in future experiments with ammonium acetate as the nitrogen source the newly determined level of 7.5 ± .5 was used.

A perusal of the terminal pH levels of the compounds listed in Table III will show that in each instance in which there was poor growth with an ammonium compound there was a correspondingly low pH value. The fact that ammonium nitrogen could be utilized by this fungus was shown by the results with ammonium acetate. Work with *Allomyces javanicus* var. *macrogynus* (MACHLIS, 1953) and with *Chytridium* sp. (CRASEMANN, 1954) illustrated that the preferential utilization of ammonium nitrogen was accompanied by a decrease in pH to an unfavorable level for growth. For this reason COCHRANE (1958) pointed out that inability of a culture to grow with ammonium nitrogen may only reflect failure to grow at a low pH. Certain ammonium and non-ammonium compounds all of which had proved to be poor sources of nitrogen at an initial pH of 6.0 (see Table III) were now investigated at pH 9.0 to

TABLE IV.

Growth of P. roseus on some nitrogen compounds at pH 9.0

Compound	Final pH	Mycelial weight g/100 ml	Change of weight from pH 6.0
NH_4HCO_3	8.1, 8.1	0.335	— 0.169
choline citrate	7.5, 7.5	0.154	— 0.111
ammonium lactate	7.3, 7.1	0.615	+ 0.135
ammonium citrate	7.1, 7.0	0.543	+ 0.031
$Ca(NO_3)_2$	7.6, 7.6	0.580	+ 0.046
ammonium acetate.	7.4, 7.5	0.850	— 0.208
$(NH_4)_2HPO_4$.	4.9, 5.0	0.743	+ 0.573
NH_4NO_3	4.4, 4.5	0.437	+ 0.260
$(NH_4)_2SO_4$.	4.1, 4.1	0.401	+ 0.062

elucidate the pH effect. After an 82 hour incubation period the results were those shown in Table IV. The results showed no marked increase in growth at an initial pH of 9.0 compared to pH 6.0, which indicated that failure of *P. roseus* to utilize these compounds was not due to a pH effect, with the possible exception of $(NH_4)_2HPO_4$.

E. Requirements for phosphate, sulfate and trace elements

Simultaneously with the work on the carbon and nitrogen nutrition an experiment was devised to establish the optimum requirements for inorganic phosphate and sulfate. The medium contained 1.5 % soluble starch and 0.4 % ammonium acetate at a pH of 7.5. Various levels of K_2HPO_4 and $MgSO_4.7H_2O$ were added to the individual flasks prior to sterilization and they were incubated for 3½ days. The results are shown in Table V. The optimum levels are 0.1 % K_2HPO_4 and 0.2 % $MgSO_4.7H_2O$. Absence of both compounds seriously inhibited growth. Comparison of the terminal pH levels showed that the inhibition was not a function of pH.

TABLE V.

Phosphate and sulfate requirements of P. roseus.

Flask	% phosphate	% sulfate	Final pH	Mycelial weight g/100 ml
1	0.1	0.5	8.0	0.790
2	,,	,,	7.9	
3	0.2	,,	8.0	0.784
4	,,	,,	7.9	
5	0.3	,,	7.9	0.794
6	,,	,,	7.9	
7	0.0	,,	7.4	0.533
8	,,	,,	7.5	
9	0.1	0.0	7.9	0.274
10	,,	,,	7.7	
11	,,	0.05	7.9	0.623
12	,,	,,	8.0	
13	,,	0.1	7.7	0.833
14	,,	,,	7.3	
15	,,	0.2	7.2	0.877
16	,,	,,	7.7	
17	0.0	0.0	7.6	0.287
18	,,	,,	7.6	

Several experiments were conducted in which the effect of trace elements was investigated. The compounds which were tested at the indicated concentrations (milligrams per liter) included: H_3BO_3, 2.86; $MnCl_2$, 1.81; $ZnSO_4$, 0.222; $CuSO_4$, 0.079; MoO_3, 0.015; $Ca(NO_3)_2$, 59; $CoCl_2$, 0.04 and $FeSO_4$, 0.1. The addition of these compounds at the concentrations indicated did not increase mycelial production.

F. Growth factor requirements.

Yeast extract serves not only as a nitrogen source, but it also contains a variety of growth factors such as purines, vitamins, etc. Therefore, continued optimal growth in media lacking yeast extract would show that a microorganism was able to synthesize the necessary growth factor requirements. With this in view, serial transfers were made through a series of flasks which contained ammonium acetate as the sole nitrogen source, and the remaining constituents were those found in the YpSs medium. At least two days growth was permitted in each flask in the series. Following four such serial transfers the final flask in the series was used as inoculum for the basal medium composed of 3 % soluble starch, 0.6 % ammonium acetate, 0.1 % K_2HPO_4 and 0.2 % $MgSO_4.7H_2O$ at a pH of 8.0. As shown in Table VI the absence of the various growth factors present in yeast extract did not significantly retard growth. A slight stimulation of growth resulted from the addition of thiamin and biotin individually. This increased growth probably resulted from a reduced lag phase and a more rapid initiation of growth. Thus, we concluded that this fungus was able to synthesize the necessary amino acids and other growth factors from the amomnium nitrogen in the medium.

TABLE VI.

Growth factor requirements of P. roseus.

Flask	Vitamin/100 ml	Final pH	Mycelial weight g/100 ml
1	thiamin, 0.1 mg	7.3	1.060
2	,,	7.2	
3	thiamin, 0.2 mg	7.1	1.393
4	,,	7.2	
5	thiamin, 0.3 mg	7.2	1.079
6	,,	7.2	
7	biotin, 0.1 mg	7.2	1.102
8	,,	7.2	
9	biotin, 0.2 mg	7.3	1.074
10	,,	7.2	
11	biotin, 0.3 mg	7.1	1.268
12	,,	7.2	
13	thiamin, 0.1 mg, biotin, 0.1 mg	7.3	0.904
14	,,	7.6	
15	control- no vitamin	7.4	0.959
16	,,	7.1	

As a corollary to this work certain vitamins were added individually to the medium in order to determine whether they would stimulate increased mycelium production. The vitamins were added at a 0.3 % concentration and included ascorbic acid, calcium pantothenate, nicotinic acid, riboflavin and pyridoxine HCl. None of them increased the growth over the controls.

G. Temperature relationships of P. roseus

All of the preliminary studies as well as the studies heretofore discussed in this paper were conducted at 28° C. However, there was no reason to assume this was the optimum temperature for the growth of this fungus, therefore, submerged growth at 28° C was compared with that at 37° C. The medium used for this study was the YpSs medium with an initial pH of 7.0. Twenty-eight flasks were inoculated simultaneously and divided into two equal lots. At various intervals over a 4 day period two flasks were removed from each shaker and the mycelium was harvested in the usual manner. The resulting growth curves are illustrated in Figure

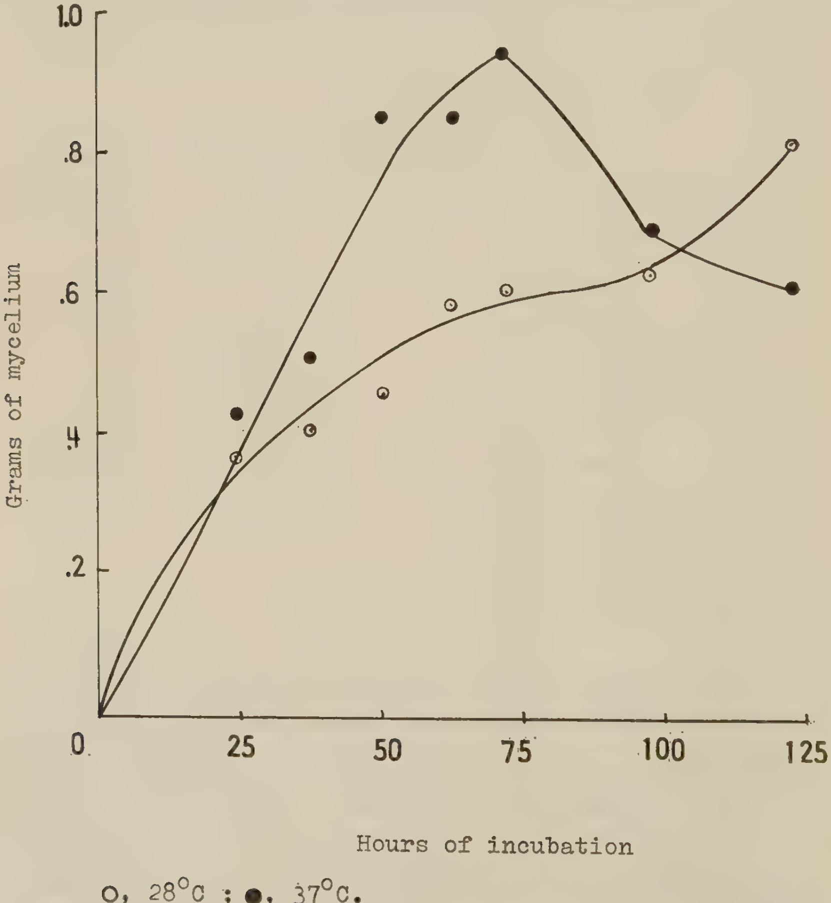


Fig. 3. Rate of growth of *P. roseus* at 28°C compared with that at 37°C.

3. It was quite apparent that at 37° C maximum mycelium was produced in less than half the time required at 28° C.

Since reciprocating shakers in incubators at other temperatures were not available, Petri dishes of YpSs medium were inoculated with the fungus and incubated at 23° C, 28° C, 37° C and 44° C. The growth rate was significantly faster at 37° C, while at 44° C growth was completely inhibited. However, when the plates which had been incubated at 44° C for 6 days were incubated further at 23° C it was found that the fungus developed normally except for an initial lag in growth when compared to control cultures. It was not surprising that the optimum temperature for the growth of this fungus was in the vicinity of 37° C. The habitat from which the mold was isolated, an ulcerated gizzard of a chicken, would indicate the pathogenic or parasitic tendencies of *P. roseus*, and the majority of fungi parasitic on animals have growth-optima around 37° C.

H. Additional information on nutrition.

Further studies with carbon sources were conducted simultaneously with those reported in the previous section on nitrogen sources. In that work it was found that the optimum source of nitrogen was ammonium acetate at a 0.6 % concentration. Therefore, with this optimum level of ammonium acetate two experiments were performed to determine the optimum levels of both soluble starch and maltose. The medium which was employed contained 0.1 % K_2HPO_4, 0.05 % $MgSO_4 \cdot 7H_2O$ and 0.6 % ammonium acetate at a pH of 6.0, plus various levels of either soluble starch or maltose. The incubation was carried out at 28° C for 5½ days. Figure 4 depicts the growth curve. Concentrations of soluble starch above 3 % did not significantly increase the weight of mycelium.

Cochrane (1958) stated that sugars have been reported to undergo a breakdown when they were autoclaved in the medium and therefore such treatment may be the cause of erroneous results in carbon utilization studies. For this reason, he maintained that sugars should never be autoclaved in the medium but may be sterilized by being autoclaved separately and added to the medium aseptically. The fact that breakdown of sugars occurred in our experiments was indicated by browning of the medium when maltose and glucose were used and autoclaved in the medium. To overcome this, as was stated earlier, soluble starch was used because it could be autoclaved in the medium with complete absence of discoloration due to decomposition.

Another factor which should be taken into consideration in nutritional investigations is the differential utilization of certain compounds by microorganisms. Therefore, growth per unit time was determined for *P. roseus* at 37° C using soluble starch, maltose, glucose which had been autoclaved in the medium and glucose which had been autoclaved separately and added to the medium

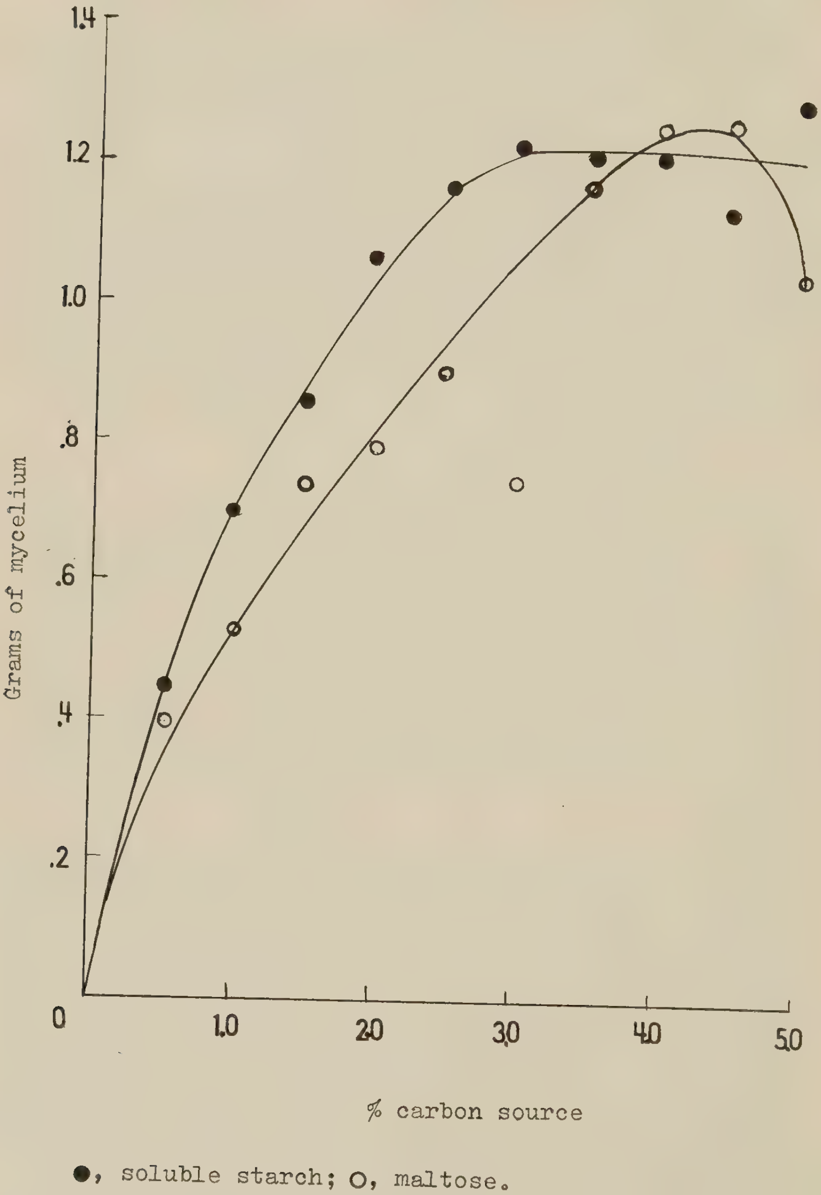
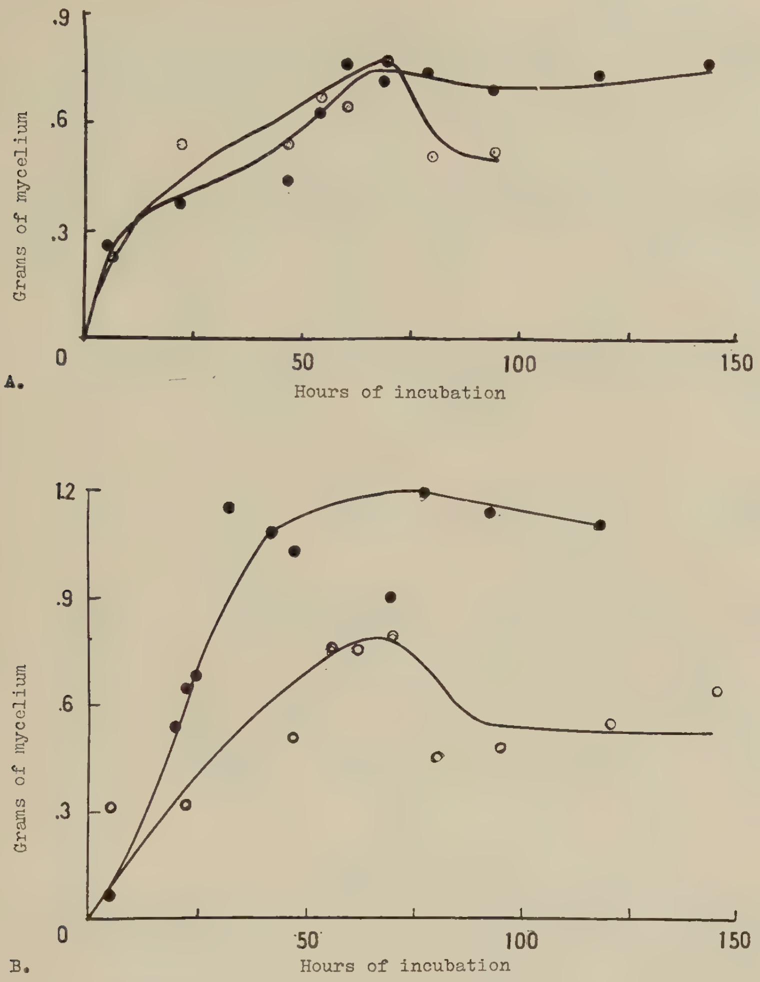


Fig. 4. Growth of *P. roseus* in relation to concentrations of soluble starch and maltose.



A. ●, glucose autoclaved in the medium; O, glucose autoclaved separately.

B. ●, soluble starch; O, maltose.

Fig. 5. Growth of *P. roseus* in an optimum medium with certain variations in the carbon source

aseptically at the time of inoculation. The medium which was used for this purpose was the optimum medium which had been developed during the course of this investigation and consisted of 3 % of the carbon source, 0.6 % ammonium acetate, 0.1 % K_2HPO_4 and 0.2 % $MgSO_4 \cdot 7H_2O$ with a pH of 8.0. The results shown in Figure 5 illustrate that the best carbon source in conjunction with ammonium acetate was soluble starch and that maximum mycelial production was attained in 32 hours as compared with 50 hours for the YpSs medium at this temperature. Thus, whereas soluble starch was inferior to maltose as a carbon source when yeast extract was the nitrogen source, the situation was reversed with ammonium acetate as the source of nitrogen.

Comparison of the growth curves for glucose autoclaved in the medium and glucose autoclaved separately showed that while maximum growth was attained slightly sooner with glucose autoclaved in the medium, the final maximum values were approximately identical. Therefore, we may assume that autoclaving sugars in the medium did not impede growth of *P. roseus*.

Table VII.

Growth of P. roseus in natural products rich in fatty acids.

Flask	Carbon source	Final pH	Mycelial weight g/100 ml
1	corn oil	7.9	1.301
2	,,	7.9	
3	sperm oil	7.9	1.360
4	,,	7.9	
5	sesame oil	7.9	1.243
6	,,	7.9	
7	cottonseed oil	8.0	1.240
8	,,	7.9	
9	olive oil	7.9	1.046
10	,,	8.0	
11	rice oil	8.2	1.345
12	,,	8.1	
13	fish oil	6.1	0.132
14	,,	6.1	
15	maltose control	7.4	0.949
16	,,	7.3	
17	soluble starch control	7.2	1.001
18	,,	7.5	
19	controls (no	8.7	0.288
20	carbon source)	8.8	
21	,,	8.8	0.295
22	,,	8.8	

As Johnson (1957) recently pointed out, the ability of molds to utilize carbon from fatty acids is not unique. At this time we wish to report a simple experiment designed to supply some information in this regard. The fungus was grown in the following medium: 0.6 % ammonium acetate, 0.1 % K_2HPO_4 and 0.2 % $MgSO_4 \cdot 7H_2O$ at a pH of 7.0. To each of the flasks some natural product rich in fatty acid content was added at a concentration of 2 %.

As was indicated previously, the carbon content of the various nitrogen sources was not taken into consideration. However, the best source of nitrogen, ammonium acetate, did contain a potential source of carbon which might have accounted for the increased mycelial production. Therefore, in this experiment four control flasks were included with no carbon source other than that offered in the ammonium acetate.

Table VII presents the results of this experiment which was incubated at 28° C for 3½ days. The maximum yields of mycelium which have been obtained throughout this study using ammonium acetate and soluble starch rarely exceeded 1.1 g/100 ml. It appeared that certain of the natural oils were at least equivalent to soluble starch and one or two were slightly superior. To determine whether the fungus utilized certain fatty acids from the natural oils the following compounds were used as sole sources of carbon at 0.5 % levels: glycerine, sodium oleate, sodium lauryl sulfate, as well as the following acids: acetic, propionic, caproic, caprylic, myristic, stearic, oleic, linoleic, valeric and isovaleric. Absolutely no growth resulted with most of the compounds while only a very small amount of mycelium was produced with sodium oleate, sodium lauryl sulfate, myristic acid and stearic acid. However, it was possible that other, untested fatty acids present in the natural oils were responsible for the increased growth of the fungus. Also, oils contain a variety of growth factors which might also have stimulated growth. Growth in the control flasks was sufficiently limited to allow us to conclude that the carbon which was contained in ammonium acetate was inadequate to support normal mycelium production. The utilization of fatty acids by certain Gymnoascaceae will be reported upon at another time.

Summary

Mycelial production by *Pseudoarachniotus roseus* KUEHN was determined when cultured in shaken flasks on a reciprocating shaker with 100 ml of various media per 500 ml Erlenmeyer flask. To measure growth the contents of each flask were centrifuged, washed and recentrifuged in order to remove residual, solid constituents of the medium. The mycelium was washed quantitatively into tared aluminum weighing cups and dried to a constant weight in an oven. Mycelium production was determined with thirty different carbon compounds and 43 different nitrogen sources which were supplied singly. Maltose, dextrin, xylose and soluble starch were the carbohydrates which permitted maximum growth, while ammonium acetate and asparagine were the best nitrogen sources. The best initial pH ranged from 6.0-9.0, while with optimum growth conditions the terminal pH was 7.0-8.0. Using soluble starch as the carbon source and ammonium acetate as the nitrogen source, maximum mycelial weights were obtained in 32 hours at 37° C and in 88 hours at 28° C. When grown in Petri dishes at 23° c,

28° C, 37° C and 44° C, *P. roseus* achieved maximum growth at 37° C, while growth was inhibited completely at 44° C.

Soluble starch was selected as the carbon source for most of the experiments because it could be autoclaved in the medium with no carmelization or breakdown. The optimal medium contained 3 % soluble starch, 0.6 % ammonium acetate, 0.1 % K_2HPO_4 and 0.2 % $MgSO_4 . 7H_2O$. Addition of low concentrations of manganese, zinc, copper, molybdenum, calcium, cobalt, chlorine, iron and boron to the basal medium did not stimulate mycelium production. No further growth requirements were exhibited by this fungus although addition of 0.2 mg % thiamin or 0.3 mg % biotin stimulated formation of mycelium. Certain natural oils stimulated growth due to undetermined factors.

Literature Cited

Beaven, G. H. & F. Brown, (1949) The pectic enzymes of the mould *Byssochlamys fulva*. Biochem. J. (Lond.) **45**: 221—224.

Berthelot, M. (1899) Chimie végétal et agricole. Tome I. Fixation de l'azote libre sur la terre et sur les végétaux. Paris.

Cochrane, V. W. (1958) Physiology of fungi. Wiley, New York. 524 pp.

Crasemann, J. M. (1954) The nutrition of *Chytridium* and *Macrochytrium*. Amer. J. Bot. **41**: 302—310.

Hawker, L. E. (1936) The effect of certain accessory growth substances on the sporulation of *Melanospora destruens* and of some other fungi. Ann. Bot. **50**: 699—719.

Heinze, B. (1906) Sind Pilze imstande, den elementaren Stickstoff der Luft zu verarbeiten und den Boden an Gesamtstickstoff anzureichern. Ann. Myc. **4**: 41—63.

Holden, M. (1950) A study of enzymes that can break down tobacco-leaf components. 3. Fungal polygalacturonase on leaf fibre. Biochem. J. (Lond.) **47**: 415—420.

Hull, R. (1939) Study of *Byssochlamys fulva* and control measures in processed fruit. Ann. appl. Biol. **26**: 800—822.

Johnson, G. T. (1957)) Fatty acids as carbon sources for the growth of *Spicaria violacea*. Mycologia **49**: 172—177.

Karow, E. O. & J. W. Foster, (1944) An antibiotic substance from species of *Gymnoascus* and *Penicillium*. Science **99**: 265—266.

Kluyver, A. J. & L. H. C. Perquin, (1933) Zur Methodik der Schimmelstoffwechseluntersuchung. Biochem. Z. **166**: 68—81.

Kuehn, H. H. (1955a) Observations on Gymnoascaceae. I. *Myxotrichum uncinatum* and a new species of *Myxotrichum*. Mycologia **47**: 533—545.

—, (1955b) Observations on Gymnoascaceae. II. Two new species of *Myxotrichum*. Mycologia **47**: 878—890.

—, (1956) Observations on Gymnoascaceae. III. Developmental morphology of *Gymnoascus reessii*, a new species of *Gymnoascus* and *Eidamella deflexa*. Mycologia **48**: 805—820.

—, (1957a) Observations on Gymnoascaceae. IV. A new species of *Arachniotus* and a reconsideration of *Arachniotus trisporus*. Mycologia **49**: 55—67.

—, (1957b) Observations on Gymnoascaceae. V. Developmental morphology of two species representing a new genus of the Gymnoascaceae. Mycologia **49**: 694—706.

Machlis, L. (1953) Growth and nutrition of water molds in the subgenus Euallomyces. II. Optimal composition of the minimal medium. Amer. J. Bot. **40**: 450—460

NYUKSHA, Y. P. (1953) A physiological study of the fungus *Gymnoascus setosus* Eidam. I. Mikrobiologiya **22:** 15—22.

—, (1955) Some reasons for spoilage of books. Nature (Moscow) **44:** 94—96.

—, (1957) A physiological study of the fungus *Gymnoascus setosus* Eidam. II. Mikrobiologiya **26:** 309—315.

RAISTRICK, H. & G. SMITH, (1933) Studies in the biochemistry of micro-organisms. XXXV. The metabolic products of *Byssochlamys fulva* Olliver and Smith. Biochem. J. (Lond.) **27:** 1814—1819.

REID, W. W. (1950) Pectic enzymes of the fungus *Byssochlamys fulva*. Nature, Lond. **166:** 76—77.

—, (1952) The pectic enzymes of the fungus *Byssochlamys fulva*. Biochem. J. (Lond.) **50:** 289—292.

ROBBINS, W. J. & V. KAVANAGH, (1942) Vitamin deficiencies of the filamentous fungi. Bot. Rev. **8:** 411—471.

SCHOPFER, W. H. & S. BLUMER, (1940) Recherches sur la répartition de l'heterotrophie par rapport à l'aneurine chez les champignons. Arch. Microbiol. **11:** 205—214.

INHIBITION OF *HISTOPLASMA CAPSULATUM* AND *BLASTOMYCES DERMATITIDIS* BY *PSEUDOMONAS AERUGINOSA IN VITRO*¹)

by

PETER FEDORS²)

(*Veterans Administration Hospital, Outwood Station, Dawson Springs, Kentucky*)

(13.II.1959)

Pseudomonas aeruginosa has long been known to produce substances which are antagonistic to many microorganisms; however, a search of the literature did not reveal any publications mentioning its antibiotic effect against *Histoplasma capsulatum* and *Blastomyces dermatitidis*. EMMERICH & LÖW, (3) in 1899, found that a filtrate from old broth cultures of *P. aeruginosa* produced an antibiotic substance. They extracted the substance and found that it had a bactericidal and lytic action on cultures of gram-positive cocci, *Neisseria*, *Corynebacterium*, *Proteus*, *Brucella*, and *Clostridium*. They ascribed this effect to the action of an enzyme which they termed "pyocyanase". The antibiotic substances were later found to resist boiling for several hours and were not enzymes but a pigment, pyocyanin, and other heat-stable substances.

CHATTON (2) found that saline extracts from *P. aeruginosa* cultures exerted a cytolytic effect on different protozoa.

SCHOENTAL (5) isolated three antibacterial substances from the chloroform extracts of *P. aeruginosa* cultures. The three substances were: (1) pyocyanin, the blue pigment which occurs in young cultures during the maximal growth of bacteria; (2) a yellow pigment identified as alpha-oxyphenazine; and (3) a colorless, chloroform-soluble, and bacteriolytic substance believed to be an acid. The second substance is a derivative of pyocyanin and was obtained in crystalline form from older cultures. The third substance was obtained from dried broth cultures which were two to three months old. This substance, oily in nature, was particularly effective against *Vibrio cholerae*. All three of the substances had a bactericidal action towards *Staphylococcus aureus* (*Micrococcus pyogenes* var. *aureus*) and *V. cholerae*.

---

¹) Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are the results of his own study and do not necessarily reflect the opinion of the Veterans Administration.

²) Present address: V. A. Hospital, Atlanta, Georgia.

SCHOENTAL performed toxicity tests for the three substances on white mice. The minimal lethal dose of the pyocyanin with intraperitoneal injection was about 2 mg. The toxicity of the alpha-oxyphenazine was relatively low. Because of its low solubility, a suspension of 10 mg. was injected intraperitoneally; it was not lethal for mice. The oily substance had a minimal lethal dose of about 5 mg. per mouse when injected intravenously; however, this amount was not lethal when injected by other routes.

The toxic action of the three substances was also tested on chicken heart fibroblasts in tissue cultures. As with the mouse toxicity tests, the pyocyanin had the greatest toxicity. The oily bacteriolytic substance was least toxic towards the chicken fibroblasts; it did not inhibit the growth of fibroblasts when it was present at a concentration of 0.3 mg./ml.

HUGHES & KIERNAN (4) found that a product of *P. aeruginosa* was active *in vitro* against *Mycobacterium tuberculosis.*

YOUNG (7) obtained an additional water-soluble pigment from chloroform extracted and ether extracted residues of *P. aeruginosa* cultures. He also obtained pyocyanin, alpha-oxyphenazine, and an antibiotic oily substance which was acid in nature, but he did not use chloroform to extract the acid-like oily substance as SCHOENTAL did. The acidlike oily substance was extracted with ether from cultures which were made acid after the extraction with chloroform. *Mycobacterium smegmatis* and *S. aureus* were sensitive to all of the four fractions; however, *Escherichia coli* was sensitive only to the pyocyanin fraction.

MATERIALS AND METHODS

The discovery of the antibiotic effect of *P. aeruginosa* on *H. capsulatum* was accidental. A series of five sputum specimens were collected from a patient suspected of having a pulmonary fungus infection. The specimens were collected on consecutive work days and each specimen was inoculated routinely on four tubes of media and incubated at room temperature. Two of the tubes contained nutrient blood agar media with antibiotics. One of the two blood agar tubes contained 20 units of penicillin and 40 μg. of streptomycin per ml. of blood agar; the other tube contained the same amounts of penicillin and streptomycin but 300 μg. of actidione were also added for each ml. of media. The third tube contained Littman's oxgall agar and the fourth tube contained Sabouraud's glucose agar.

Extraction and Testing of Antibiotic Substances

A medium of 500 ml. of brain heart infusion broth containing 2 % glycerol was used to grow the antibiotic-producing *P. aeruginosa.* The culture was incubated at room temperature for seven days and killed by heating at 56 C. for 4 hours.

The pyocyanin, alpha-oxyphenazine, and other chloroform soluble substances were extracted from the broth and organism mixture by adding 200 ml. of chloroform.

The pyocyanin was extracted from the chloroform fraction by adding 100 ml. of 0.05 *N* HCl. The pink, acid solution of pyocyanin was neutralized to a blue color with NaOH and concentrated by boiling to an optical density of 0.35 for light with a wavelength of 610 mμ. This concentrated pyocyanin solution was used in making the dilutions incorporated in the test media.

The chloroform fraction, containing alpha-oxyphenazine, was evaporated and 0.12 Gm. of a yellow-brown, partially crystalline substance was obtained. This substance was also oily in nature, had an odor characteristic of *Pseudomonas* cultures and may have contained some of the oily chloroform-soluble substances mentioned by SCHOENTAL. The material was soluble in 4 ml. of 0.2 *N* NaOH but the solution solidified upon being placed in a refrigerator. When the alkaline solution was made acid a white precipitate formed. The precipitate rose to the surface when the acid mixture was centrifuged. A microscopic examination of the precipitate revealed yellow crystals. The alkaline solution was used in preparing the dilutions incorporated in the test media.

The chloroform-extracted residue was made acid with 5 ml. of *N* HCl and extracted with 50 ml. of ether to obtain the oily acid-like substances mentioned by SCHOENTAL and YOUNG. About 0.075 Gm. of an oily yellow-brown substance was obtained from the ether fraction. This substance was also soluble in chloroform. This fraction differed from the chloroform-extracted fraction in that not all of the material was soluble in 0.2 *N* NaOH and the alkaline solution did not gel when placed in the refrigerator. When the alkaline solution was acidified, a white precipitate formed which was heavier than the acid solution. There were no crystalline structures in the precipitate; however, a yellow amorphous material containing darker yellow globules was present. It is possible that the yellow globules represented residual alpha-oxyphenazin which could not crystallize because of oily impurities. Only the alkali-soluble material was used in preparing the dilutions incorporated in the test media.

Four-fold dilutions of the three different solutions were made and 1 ml. of each dilution were added to tubes of melted Sabouraud's agar before the tubes were allowed to slant. In order to keep the chloroform- and ether-extracted substances in solution, the dilutions were made in 0.2 *N* NaOH and each tube of media was neutralized after the addition of the alkaline dilutions.

The antibiotic effects of various dilutions of the above fractions were tested on cultures of *H. capsulatum*, *B. dermatitidis*, *Cryptococcus neoformans*, *E. coli*, and *S. aureus*. The culture of *S. aureus* was isolated from the patient from whom the antagonistic cultures of *H. capsulatum* and *P. aeruginosa* were obtained. In addition to the above organisms, the chloroform-extracted fraction was also tested with cultures of *Sporotrichum schenckii*, *Geotrichum candidum*, and *Candida albicans*.

TABLE I.

*Number and Identity * of Colonies Grown from Various Specimens at Seven Days*

Medium	Specimen Number				
	1	2	3	4	5
Blood agar with penicillin, streptomycin, and actidione	Confluent colonies of *H. capsulatum*, 6 *S. aureus*	1 *P. aeruginosa*, confluent colonies of *H. capsulatum*, ** 3 *S. aureus* ***	15 *P. aeruginosa*	20 *P. aeruginosa*	nearly confluent colonies of *P. aeruginosa*
Blood agar with penicillin, and streptomycin	Confluent colonies of *H. capsulatum* 7 *S. aureus*, 1 *Penicillium* sp.	2 *P. aeruginosa*, confluent colonies of *H. capsulatum*, ** 3 *S. aureus*, 1 *Aspergillus* sp.	10 *P. aeruginosa*, 5 *S. aureus*	25 *P. aeruginosa*	nearly confluent colonies of *P. aeruginosa*
Littman's oxgall agar	no growth	3 *P. aeruginosa* 1 *Mycelia sterila*	30 *P. aeruginosa*	confluent colonies of *P. aeruginosa*	confluent growth of *P. aeruginosa*
Sabouraud's glucose agar	200 *S. aureus*	1 *P. aeruginosa*, 200 *S. aureus*	30 *P. aeruginosa* 20 *S. aureus*	1 *Aspergillus niger*, 50 *P. aeruginosa*	nearly confluent colonies of *P. aeruginosa*

*) Some of the colonies were actually identified after seven days, and most were identified by appearance only.

**) Colonies did not appear within 0.5 cm. from the colonies of *P. aeruginosa*.

***) Some colonies were in contact with the colonies of *P. aeruginosa*.

RESULTS

The growth obtained from the various specimens on the different media can be seen on table I.

Cultures from the first specimen showed numerous colonies of *H. capsulatum* on the blood agar slants. The second specimen also revealed numerous *H. capsulatum* colonies on both of the blood agar slants; however, 1 colony of *P. aeruginosa* was present on one of the blood agar slants, and 2 colonies were present on the other blood agar slant. Three colonies and 1 colony of *P. aeruginosa* were present on the Littman's oxgall agar and the Sabouraud's glucose agar respectively. There were no *H. capsulatum* colonies within 0.5 cm. of the colonies of *P. aeruginosa* on the blood agar slants.

None of the specimens taken on the third, fourth, and fifth day revealed any *H. capsulatum*; however, the colonies of *P. aeruginosa* appeared in increasing numbers.

Although *P. aeruginosa* was partially susceptible to the streptomycin in the blood agar media, the colonies were nearly confluent in cultures from the specimens taken on the fifth day.

Experiments to confirm the results and to determine the inhibition of other systemic fungi.

To make sure that *H. capsulatum* was not absent from the last three specimens, a check was made. A colony of *H. capsulatum* (isolated from another patient) was macerated and inoculated onto 3 tubes of nutrient blood agar in such a way as to obtain confluent growth over the entire slant. *P. aeruginosa* organisms were inoculated onto one of the slants along a line extending from the center of the slant to the bottom of the slant. A culture of *S. aureus* was inoculated in a similar manner on one of the two remaining slants. Except for two small colonies of *H. capsulatum* appearing on the top of the slant containing the *P. aeruginosa*, the growth of the fungus was completely inhibited. Both of the control tubes had confluent growth of *H. capsulatum* over the entire slant, except in the area covered by the faster growing *S. aureus* organisms in one of the tubes.

By a similar method, cultures of *B. dermatitidis*, *S. schenckii*, *C. neoformans*, *G. candidum*, and *C. albicans* were inoculated to see if they were sensitive to the same culture of *P. aeruginosa*. The growth of *B. dermatitidis* was inhibited much as the *H. capsulatum* was inhibited, but the growth of *S. schenckii*, *C. neoformans*, *G. candidum*, and *C. albicans* did not appear to be inhibited under the conditions of the experiment. However, it was found from another patient, that a *Pseudomonas* species which did not liquify gelatin reduced the rate of growth of a species of *Candida*. The organisms were obtained from a sputum specimen and were growing on Littman's oxgall agar when the observation was made.

Antibiotic Effect of the Three Fractions

Pyocyanin (Water-Soluble) Fraction. Except for a slight inhibition of the *H. capsulatum*, none of the fungi were inhibited by a concentration of $4^{-2,5}$ ml. of pyocyanin solution per ml. of media.

Chloroform-Extracted (Alpha-oxyphenazine containing) Fraction. The growths of *H. capsulatum* and *B. dermatitidis* were completely inhibited by concentrations of 4^{-7} Gm. and 4^{-4} Gm. respectively per ml. of media. There was also partial inhibition of these fungi at the next higher dilution (One-fourth the concentration). The cultures of *C. neoformans*, *S. schenckii*, *G. candidum*, *C. albicans*, *S. aureus*, and *E. coli* were not inhibited by a concentration of 4^{-4} Gm. per ml. of media.

Oily, Ether-Extracted Fraction. The growths of *H. capsulatum* and *B. dermatitidis* were completely inhibited by concentrations of 4^{-6} Gm. and 4^{-4} Gm. respectively per ml. of media. There was also partial inhibition of these organisms at the next higher dilution. The cultures of *C. neoformans*, *S. aureus*, and *E. coli* were not inhibited by a concentration of 4^{-4} Gm. per ml. of media.

Discussion

Since present antimycotic agents are few and not very reliable, it may be advantageous to determine whether any of the antibiotic substances of the *Pseudomonas* species could be used for the therapy of fungus diseases. Older cultures of *P. aeruginosa* produce hemolysins; however, in the above experiments, the fungus inhibiting substance appears before the hemolysis of the blood agar is noticeable. It would also be advisable to investigate the genus *Xanthomonas* for antimycotic substances.

Bouchard (1), in 1880, demonstrated that rabbits injected with anthrax bacilli could be prevented from developing the disease by inoculation with the "blue pus" bacillus.

Pyocyanin and alpha-oxyphenazine from *P. aeruginosa* cultures have been used for the biochemical treatment of patients with schizophrenia or psychoses (Schuster) (6).

It will be interesting to follow the progress of the patient from whom the *H. capsulatum* and *P. aeruginosa* organisms were isolated. It is also possible that *Mycobacterium tuberculosis* may be inhibited *in vivo*, or killed *in vitro*, by the presence of *P. aeruginosa*. A patient who had continuously been positive on cultures for *M. tuberculosis* in sputum had converted to negative at about the time during which a culture of a *Pseudomonas* species was isolated from a sputum specimen.

The culture of *P. aeruginosa* mentioned was resistant to all antibiotics tried except for partial sensitivity to streptomycin and dihydrostreptomycin. Because of the resistance of the *Pseudomonas* species to antibiotics ordinarily used in fungus media, they are of diagnostic importance in the isolation of pathogenic fungi.

SUMMARY

It was accidentally discovered that *Pseudomonas aeruginosa* inhibited the growth of *Histoplasma capsulatum* when both of the organisms were isolated from a series of sputum specimens obtained from a single patient. Experiments were performed to determine if there is any inhibitory effect on other systemic fungi.

Three fractions were obtained from a broth culture of *P. aeruginosa* and their antibiotic effects tested against various systemic fungi and bacteria.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Elizabeth Ulrich the bacteriologist from whom the stock culture of *B. dermatitidis* was obtained, to Dr. Henry A. Bok who ordered the fungus studies on the patient, and to Dr. Kenneth L. Lockwood for his continued interest in mycological studies.

References

1. Bouchard, C. (1889) Influence qu'exerce sur la maladie charbonneuse l'inoculation du bacille pyocyanique, Compt. rend. **108:** 713—714.
2. Chatton, E. & M. (1927) Properties and nature of cytolysin of *Bacillus pyocyaneus,* Compt. rend. Soc. de biol. **97:** 292—295.
3. Emmerich, R. & Löw, O. (1899) Bakteriologische Enzyme als Ursache der erworbenen Immunität und die Heilung von Infektionskrankheiten durch dieselben, Z. Hyg. Infektionskrankh. **31:** 1—15.
4. Hughes, G. C. & Kiernan, R. A. (1947) Product of *Pseudomonas aeruginosa* active *in vitro* against *Mycobacterium tuberculosis,* Nature, London, **159:** 197.
5. Schoental, R. (1941) The nature of the antibacterial agents present in *Pseudomonas pyocyaneus* cultures, Brit. J. exper. Path. **22:** 137—147.
6. Schuster, J.: Chemical background of pyocyaneus therapy of schizophrenia and psycoses (Wagner-Jauregg Method), Arch. f. Psychiat. **108:** 120—218.
7. Young, G. (1947) Pigment production and antibiotic activity in cultures of *Pseudomonas aeruginosa,* J. Bact. **54:** 109—117.

SCHEDE MICOLOGICHE
III CONTRIBUTO
MICROMICETI DELLA VALLE DEL TEVERE [1]

ANGELO RAMBELLI

(Centro di Sperimentazione Agricola e Forestale dell'Ente Nazionale per la Cellulosa e per la Carta Roma)
Direttore: Prof. ALESSANDRO DE PHILIPPIS

(11.III.1959)

Da quando abbiamo iniziato la pubblicazione delle nostre "Schede micologiche" (10—11), ci siamo preoccupati di descrivere solo quei microrganismi che avevano possibilità di sviluppo sui substrati artificiali. Questo per poter rendere noti anche i caratteri degli apparati vegetativi di quei funghi, che sui substrati naturali, normalmente non formano micelio. Questo modo di agire ci ha indotti ad accantonare e a trascurare tutte quelle forme che non crescevano o crescevano stentatamente sui comuni substrati da coltura. Abbiamo cosi perduto molto materiale interessante, che poteva essere reso noto facilmente, mediante la descrizione dei caratteri morfologici rilevabili.

Da oggi cercheremo di identificare anche quei microrganismi che non crescono sui comuni substrati da coltura, o crescono stentatamente, o il cui isolamento per le spesso scarse disponibilità di materiale da studiare, non è conveniente, Per detti microrganismi, trascureremo la descrizione degli apparati vegetativi, limitandola ai casi in cui tale forma è presente in natura, o facilmente ottenibile artificialmente. Grande rilievo daremo invece agli apparati riproduttivi, rinvenuti sulle matrici.

In questi casi le nostre "Schede micologiche" comprenderanno i seguenti punti:

Origine del materiale
Caratteri dell'apparato riproduttivo
Caratteri del microrganismo sul substrato naturale
Identificazione

Nel presente lavoro descriviamo i caratteri tassonomici di tre ifali non isolati in colture artificiali e rinvenuti su legno morto di *Populus alba* L., raccolto nel Lazio, sulle rive del fiume Tevere.

---

[1] Il lavoro è stato eseguito presso la Sezione di Protezione Fitosanitaria, sotto la guida del Consulente Prof. C. SIBILIA.

DEPHILIPPIA HELICOTRICHOIDES N. GEN. ET SP.

Origine del materiale

Rinvenuto, nel dicembre 1958, su legno morto di *Populus alba* L. Valle del Tevere — Lazio — Italia Centrale.

Caratteri dell'apparato vegetativo sul substrato naturale

Il micelio è composto da ife ialine o giallo chiare quando invecchiano, larghe da 2,50 a 3 micron, a setti e pareti difficilmente visibili, a contenuto protoplasmatico omogeneo, a calibro costante e regolare.

Le ife si riuniscono spesso in fascetti di tre o quattro elementi, larghi da 5 a 6 micron. Talvolta formano sulla superficie del substrato naturale sorta di ifenchimi, nascosti dalle fruttificazioni conidiche del fungo. Lungo il decorso delle ife, che hanno generalmente andamento strisciante, e da queste prodotti, sorgono dei falsi conidiofori, o pseudoconidiofori, che si innalzano dal substrato con forme svariatissime. Si tratta di elementi setoliformi, di colore bruno, bruno scuro, inizialmente eretti, ma con gli apici incurvati a forma di falce, o di spirale. Misurano da 120 a 170 micron in lunghezza. Verso la base si allargano fino a 15 micron e verso l'apice si attenuano, anche di colore. Sono composti da quattro — sei cellule regolari, a pareti e setti facilmente visibili e a contenuto protoplasmatico omogeneo. Le pareti sono lisce, ma divengono leggermente echinulate verso l'apice. Tali elementi, non sono in grado di produrre conidi.

Caratteri dell'apparato riproduttivo

Conidiofori ialini, lunghi da 5 a 7,50 micron, larghi da 2,50 a 3 micron, fialidiformi, a base rigonfiata e apice attenuato, a contenuto protoplasmatico omogeneo e a pareti difficilmente visibili. Sono portati in grande numero dalle ife miceliche, particolarmente in prossimità dei pseudoconidiofori. Sono in grado di produrre 15 o 20 ed anche più fialospore.

Queste ultime sono ialine, allungate, misurano da 12 a 15 micron in lunghezza e 2,50 micron in larghezza, bastoncelliformi, con un apice leggermente arrotondato, e l'altro, per mezzo del quale si inseriscono sul fialide, attenuato. Sono monocellulari, a pareti difficilmente visibili e a contenuto protoplasmatico generalmente omogeneo. Rimangono unite a fruttificazione avvenuta, all'apice del fialide, per mezzo di una sostanza mucillagginosa.

Raramente è possibile constatare la presenza di sorta di conidiofori, che prendono origine, come i pseudoconidiofori, dalle ife miceliche. Sono giallo chiari a setti e pareti facilmente visibili, a contenuto protoplasmatico omogeneo. Misurano da 12 a 15 micron in lunghezza e da 3 a 6 micron in larghezza. All'apice producono conidi, attraverso sorta di sterigmi che si formano successivamente con l'allungamento del conidioforo. Questi conidi presentano le stesse caratteristiche delle fialospore precedentemente descritte.

Caratteri delle colonie nell'ambiente naturale

Forma colonie composte da numerosissimi elementi, feltrose, di pochi millimetri di diametro e non visibili macroscopicamente. Predilige le zone decorticate.

Identificazione

Il microrganismo descritto, presenta indubbie parentele con il genere *Helicotrichum* NEES (1818). Infatti in Sylloge Fungorum (vol. IV- pag. 313) SACCARDO, accettando la diagnosi di NEES, riporta: ,,Hyphae steriles erectae, simplices apice eximie circinatae, fuliginae. Conidia bacillaria, hyalina, in basidiis brevibus ad pedem hypharum sitis acrogena."

Il nostro microrganismo però non presenta conidiofori inseriti alla base di ife setoliformi, ad apice sterile. In effetti gli organi fertili, che sono fialidi, sono portati dalle ife miceliche, sulle quali si alternano anche le ife setoliformi, ma quest'ultime sono ben distinte dai fialidi.

La specie tipica del genere, l'*Helicotrichum obscurum* (CORDA) SACC. ha conidiofori monofruttificanti, mentre il nostro è in grado di produrre venti o più conidi per conidioforo.

Noi non siamo propensi a sottovalutare questi caratteri differenziali e riteniamo più preciso istituire, per il nostro microrganismo, il nuovo genere *Dephilippia*, e la nuova specie *helicotrichoides* come tipica.

Dephilippia n. gen.

Pseudoconidiophora sterilia, saetiformia, brunea, septata, apice flectato et spiralato in micelia locata. Phyalidia fertilia, in micelia locata. Conidia esogena hyalina, cylindracea, continua, ex phyalidia exilientia.

Dephilippia helicotrichoides n. sp.

Hyphis micelis dilute bruneis, obscure septatis 2,5—3 micron crassis, protoplasmate omogeneo. Pseudoconidiophoris saetiformibus, bruneis, superne flectatis et tortilibus, inferne inflatis usque ad 15 micron, ab hyphibus micelibus productis, distincte septatis, 120—170 micron longis. Phyalidis fertilis, ab hyphibus productis 5—7 micron longis, et 2,50—3 micron crassis, hyalinis, unicellularibus, protoplasmate omogeneo. Conidiis hyalinis, homogeniis, in magna copia ex apice phyalidis exilientibus, cylindricis, unicellularibus, protoplasmate omogeneo, 12—15 micron longis et 2,50 micron crassis.

Hab. in ligno putrescente *Populi albae* L. in valle Tiberi prope Romae. Per le caratteristiche descritte, includiamo la nostra *Dephilippia helicotrichoides* nella quarta sezione della classificazione di HUGHES.

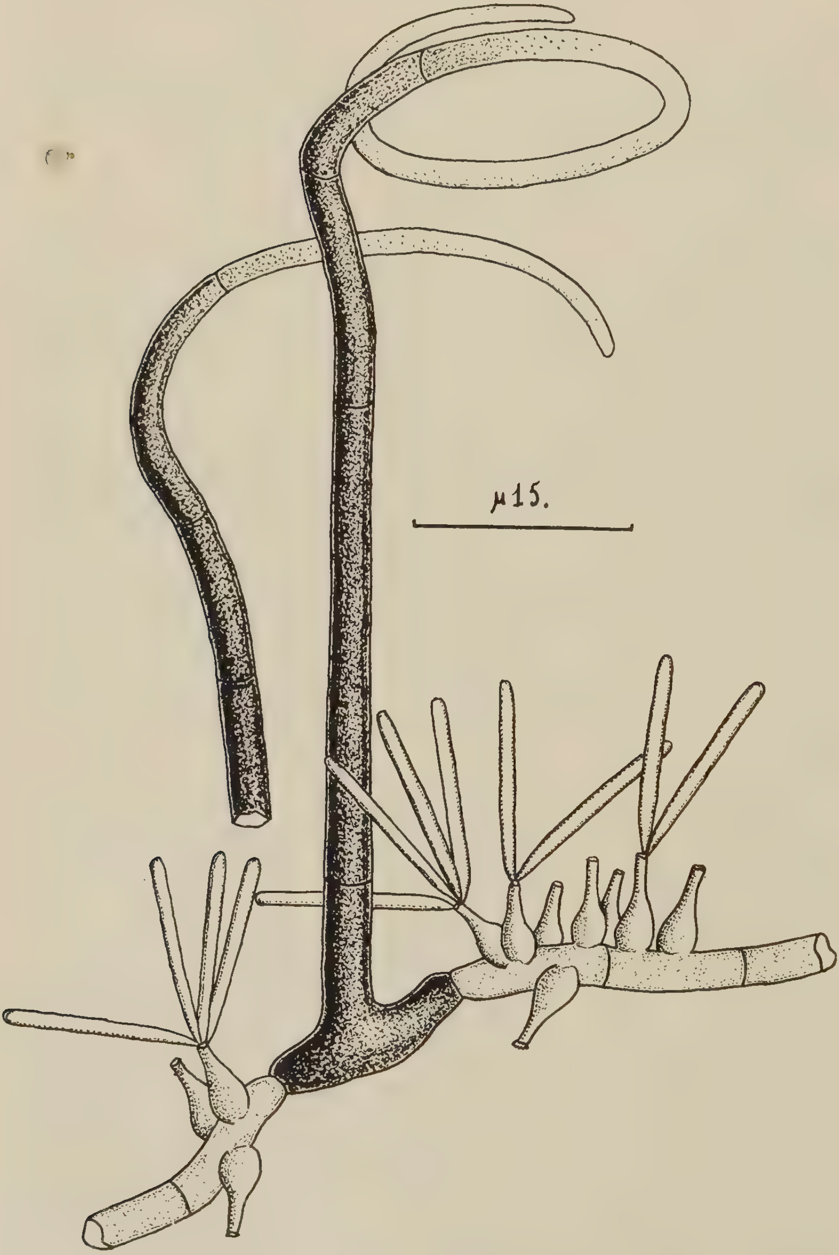


Fig. 1. *Dephilippia helicotrichoides* n. gen. et sp.

ACROTHECA SIBILIAE N. SP.

Origine del materiale

Rinvenuto, nel dicembre 1958, su legno morto di *Populus alba* L. — Valle del Tevere — Lazio — Italia Centrale.

Caratteri dell'apparato riproduttivo

Il conidioforo è formato da un asse rigido, setoliforme, ad andamento generalmente irregolare, incurvato in prossimità della base. A circa due terzi di altezza, dopo un andamento approssimativamente rettilineo, presenta una o due e talvolta più deviazioni ben rimarchevoli. E' lungo da 80 a 160 micron, composto da dieci o dodici cellule distintamente delineate, a pareti doppie e setti ben visibili, a contenuto protoplasmatico omogeneo e a colore bruno olivaceo. Tali cellule presentano talvolta rigonfiamenti ben accentuati, specialmente immediatamente sopra i setti. Verso la base si allarga fino a 10 o 12 micron, assumendo una forma leggermente globosa e una pigmentazione più intensa. Verso l'alto si attenua leggermente di colore. La fruttificazione avviene all'apice del conidioforo, attraverso punti di fuoriuscita dei conidi, sorta di sterigmi che si formano successivamente con l'allungamento del medesimo. Si viene così a formare una zona conidiogena, che da ialina diviene via via pigmentata, fino ad assumere le stesse caratteristiche cromatiche del conidioforo; ha dimensioni svariatissime, ed è composta da otto o dieci punti di differenziazione dei conidi, ognuno dei quali è in grado di produrre un unico conidio, che rimane per un certo tempo unito al punto di emissione.

Il peso dei conidi è generelamente sufficiente per determinare il ripiegamento del conidiogeno verso il basso, il quale tende però a riacquistare l'andamento primitivo durante l'accrescimento. Il ripetersi del fenomeno determina la formazione di una zona conidiogena ad andamento quanto mai irregolare.

I conidi sono ialini, o tenuamente pigmentati di giallo, bicellulari, allungati, misurano da 10 a 12,50 micron in lunghezza e 5,50 micron in larghezza, piriformi, con un apice arrotondato e l'altro, per mezzo del quale sono sostenuti sul conidioforo, appuntito. La cellula basale (appuntita) è lunga da 5 a 7 micron, l'apicale da 5 a 6 micron. Hanno pareti e setti difficilmente visibili, contenuto protoplasmatico delle cullule leggermente granuloso.

Caratteri delle colonie nell'ambiente naturale

Non forma colonie vere e proprie, ma compare a elementi isolati o sparsi, o talvolta a gruppi di due o tre. Predilige le zone decorticate dove è generalmente accompagnato da *Acrotheca humilis* RAM. e da *Acrotheca Giordani* n. sp.

Identificazione

Per i caratteri morfologici descritti, riteniamo che il microrganismo debba essere incluso nella seconda sezione della classificazione di HUGHES, sotto il genere *Acrotheca*. Non essendoci possibile alcun riferimento specifico, riteniamo debbasi istituire la nuova specie *Sibiliae*, di cui diamo le seguenti caratteristiche diagnostiche:

Acrotheca Sibiliae n. sp.

Conidiophoris setiformis, sinuosis, bruneis, distincte septatis, 80—160 micron longis, inferne usque ad 12 micron crassis, superne dilutioribus, 10—12 cellulis praeditis. Conidiogenis irregularis late flectatis, dilute bruneolis, 8—10 cicatricibus conidis praeditis. Conidiis dilute bruneolis, bicellularis, acrogenis 10—12,50 micron

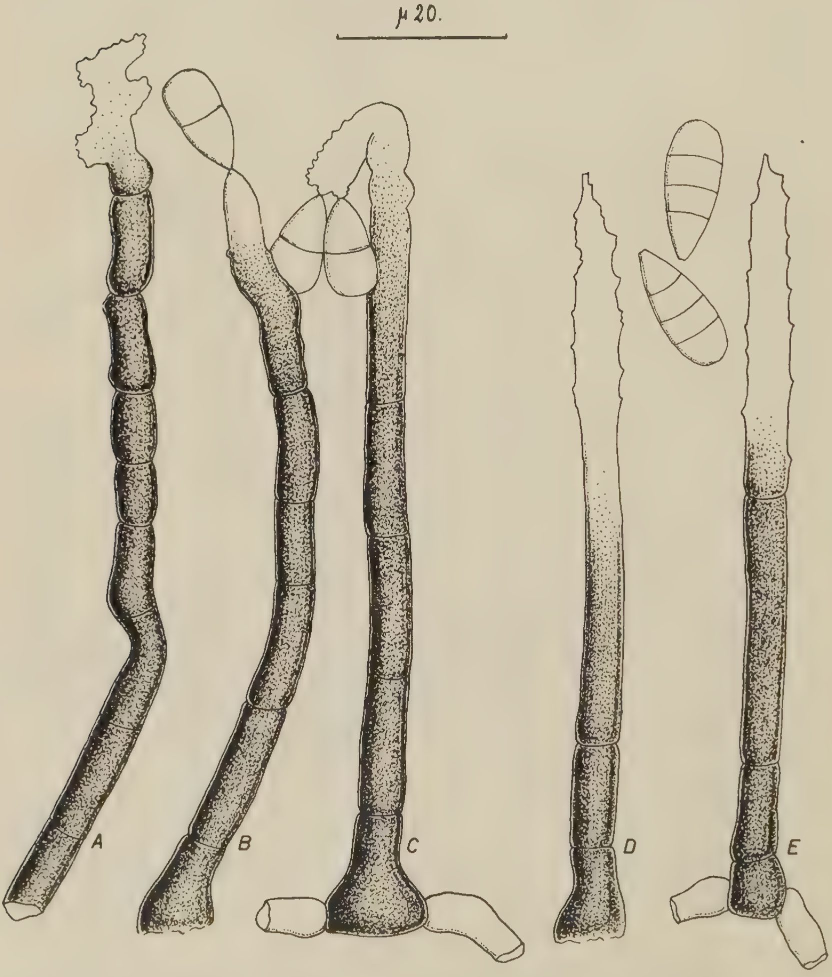


Fig. 2. *Acrotheca Sibiliae* n. sp. (A.-B.-C.); *A. Giordani* n. sp. (D-E)

longis et 5,50 micron crassis, piriformis, protoplasmate granuloso.
Hab. in ligno putrescente *Populi albae* L. in valle Tiberi prope Romae.

ACROTHECA GIORDANI N. SP.

Origine del materiale

Rinvenuto, nel dicembre 1958, su legno morto di *Populus alba* L. — Valle del Tevere — Lazio — Italia Centrale.

Caratteri dell'apparato riproduttivo

Il conidioforo è composto da un asse rigido, setoliforme, eretto, o leggermente incurvato in prossimità della base, lungo da 85 a 100 micron formato da tre o quattro cellule distintamente delineate, a contenuto protoplasmatico omogeneo, e a setti e pareti doppie facilmente visibili. Verso l'alto si assottiglia e si attenua di colore. Verso la base si allarga, in forme svariatissime fino a 6—10 micron.

La fruttificazione inizia dalla terza — quarta cellula a partire dalla base, attraverso sorta di sterigmi, che si formano all'apice del conidioforo, man mano che prosegue l'allungamento del medesimo. Si viene in questo modo a formare una zona conidiogena, più chiara del rimanente conidioforo, monocellulare e lunga da 40 a 50 micron. I punti di differenziazione dei conidi sono in numero di trenta quaranta e sono in grado di produrre un unico conidio.

I conidi sono ialini, quadricellulari, allungati, misurano da 13 a 15 micron di lunghezza e da 5 a 6 micron di larghezza, leggermente fusiformi, con un apice appuntito, per mezzo del quale si inseriscono sul conidioforo e l'altro rotondeggiante.

Hanno pareti e setti difficilmente visibili e contenuto protoplasmatico delle cellule granuloso.

Caratteri delle colonie nel substrato naturale

Non forma colonie vere e proprie, ma cresce ad elementi isolati, prediligendo le zone decorticate. E' facile trovarlo accompagnato ad *Acrotheca humilis* RAM. e ad *Acrotheca Sibiliae* RAM.

Identificazione

Per le caratteristiche descritte, riteniamo che possa essere incluso nella seconda sezione della classificazione di HUGHES, sotto il genere *Acrotheca*. Non essendoci possibile alcun riferimento specifico riteniamo debbasi per esse istituire la nuova specie *Giordani*.

Acrotheca Giordani n. sp.

Conidiophoris setiformis, bruneis, parietis et saeptis clare manifestis, protoplasmate omogeneo, 85—100 micron longis, inferne usque ad 10 micron crassis, superne dilutioribus. Conidiogenis erectis, dilutae bruneis, 30—40 cicatricibus conidicis praeditis. Conidiis hyalinis, acrogenis, quadricellularibus, fusoideis parietibus et saeptis obscure manifestis, protoplasmate granuloso, 13—15 micron longis et 5—6 micron crassis.

Hab. in ligno putrescente *Populi albae* L. in valle Tiberi prope Romae.

RIASSUNTO

Tre ifali rinvenuti su legno morto di *Populus alba* L., raccolto nel Lazio, sono identificati come nuove specie, una del nuovo genere *Dephilippia* e due del genere *Acrotheca* FUCKEL, I caratteri tassonomici riguardano gli organi vegetativi e riproduttivi sviluppati sul substrato naturale.

SUMMARY

Three Hyphomycetes found on dead wood of *Populus alba* L., collected in Lazio, have been identified as new species, one belonging to the new genus *Dephilippia* and two to the genus *Acrotheca* FUCKEL. The taxonomic characters have been represented based on the vegetative and reproductive organs developed on natural substratum.

BIBLIOGRAFIA

ARNAUD G., 1953. *Mycologie concrete. Genera II.* Bull. Soc. Mycol. France **69:** 265—306.

FERRARIS, 1910. *Flora italica Cryptogama. Hyphales.* L. Cappelli. Rocca S. Casciano.

HUGHES S. J., 1951. *Stachylidium, Gonytricum, Mesobotrys, Chaetopsis and Chaetopsella.* Trans. Brit. Mycol. Soc. **34:** 552—576.

HUGHES S. J., 1951. *Studies on Micro-Fungi. V. — Acrotheca.* Mycol. Pap. The Commonwelth Mycological Institute — Kew, Surrey. 38.

HUGHES S. J., 1953. *Conidiophores, conidia and classification.* Canad. J. Bot. **31:** 577—659.

HUGHES S. J., 1953. *Some Follicolous Hyphomycetes.* Canad. J. Bot. **31:** 560—576.

HUGHES S. J., 1958. *Revisiones Hyphomycetum aliquot cum appendice de nominibus rejiciendis.* Canad. J. Bot. **36:** 727—836.

MASON E. W., 1928. *Annotated account of fungi received at the Imperial Bureau of Mycology.* List II **1:** 1—43. The Imperial Bureau of Mycology Kew Surrey.

RAMBELLI A., 1956. *Chaetopsina nuovo genere di Ifali Demaziacei.* Atti Accad. Sci. Ist. Bologna. **III:** 191—196.

RAMBELLI A., 1957. *Schede micologiche — Micromiceti della foresta di Campigna. I Contributo.* Atti Accad. Sci. Ist. Bologna. **IV:** 189—204.

RAMBELLI A., 1958. *Schede micologiche — Micromiceti della foresta di Campigna.* II *Contributo.* Atti Accad. Sci. Ist. Bologna. **V:** 1—15.

SACCARDO P. A., 1882—1902. *Sylloge Fungorum. Sumptibus Auctoris.* Typis Seminarii Pavia.

RARE MYCOLOGICAL FINDINGS IN PATHOLOGICAL MATERIAL

by

A. N. Aravijsky, M. D.

(*Professor and Director of the Department of Dermatology, First I. P. Pavlov Medical Institute, Leningrad.*)

(12.I.1959)

One of the chief defects of everyday mycological examinations, performed in many clinics and laboratories, consists in the fact, that only a part of existing methods of examination is used, and the results which are obtained are compared merely with common signs of widely known fungi.

The thorough study of the patient's organism, the use of complex methods of mycological examination and a careful analysis of every peculiar mycological finding in the same way as the clinical casuistics, extend our knowledge of the deep character of mycotic lesions and the nature of mycoses, and hence contribute to the further development of medical mycology.

In this way I consider it useful to present the results of long investigations of deep mycotic lesions, and the systemic reactions of the organism, as the consequence of some fungal disease.

Rare mycological findings do not always correspond to rare fungal diseases. The pathological processes are known well enough, but still many things remain which are not quite clear due to the existing concept of the superficial character of most of the common mycoses.

I am going to give some examples illustrating this section of mycology.

Rare findings as the result of studying deep trichophytia

We succeeded in finding the areas of trichophytid lesions of the bones of the phalanges of three toes of the right foot. This histopathological finding was the culmination of observations on two members of the family B., which were conducted during many years. The older brother B., who was sent to us for consultation with the diagnosis Pityriasis rubra Hebrae, made by one of the clinics, was suffering from chronic trichophytia of the skin, nails and hair in the form of large, erythemat-squamous lesions, which sometimes were very like the erythrodermia of the type of Pityriasis rubra Hebrae. His younger sister B. P. was suffering from her childhood from an unrecognized chronic trichophytia: Trichophytia adultorum chronica corporis et faciei, capillitii et unguinum pedis et manus utriusque.

B. P. when a girl in her teens was injured during the cocking of the hay (the metal pitchfork fell down and pierced her foot near the base of the third toe).

After the healing of the wound a subacute inflammatory process has gradually developed on the scar. (Fig. 1). A swelling appeared, the configuration of the third, and afterwards, of the second and the fourth toes was changed. With the lapse of time an abscess and afterwards, a fistula, on the plantar side of the toe, were formed, and still later, an acute lymphadenitis of the right thigh developed, as the result of the wound of the right foot. A genuine atrophy of the bones of three toes was noticed on X-ray pictures. In the most injured third toe there was a complete destruction of the middle phalange, the external part of the first phalange. The external parts of the bones of other phalanges were also destroyed. Besides the above mentioned changes and the osteoporosis of little bones of the foot distinct endostal deposits were also noticed.

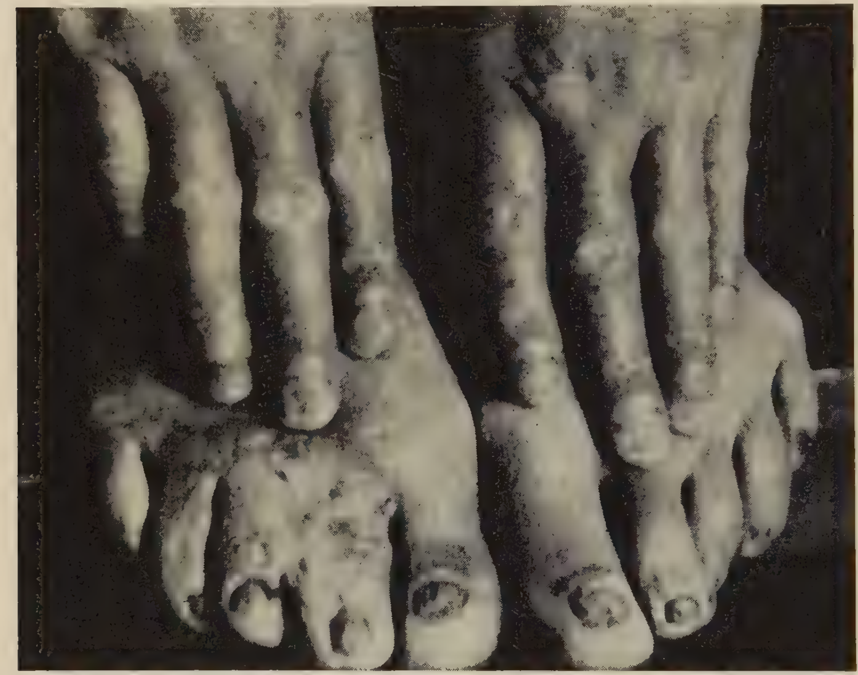


Fig. 1. Trichophytid dactylitis of 2nd, 3rd and 4th toes of right foot, onychomicosis in the patient P. B.

The complex examination of the patient according to the plan of our clinics gave the possibility to make a more detailed diagnosis and to acquire new data which supported the diagnosis trichophytia of the bones: Phalangitis-osteomyelitis chronica trichophytina digitorum II, III et IV pedis dextri (juxta cicatricem post trauma). Lymphadenitis chronica trichophytina femoralis dextra consecutiva post osteomyelitem specificam.

The results of many investigations sustained the trichophytid etiology; mycelial filaments were found in the scales on different parts of the face, body, in the hair, in the discharge of fistulae, on the plantar side of the second and third toes of the right foot, in the discharge of the fistula located in the middle of the lymphatic node in the region of the right thigh. All above mentioned pathological products when inoculated on the sugar agar of Sabouraud and other artificial media produced the colonies of *Trichophyton crateriforme* (Fig. 2 a, b, c, d, e, f, g,). To support the idea of the trichophytid etiology of the osteomyelitis, histopathological methods were used: it was possible to utilize these methods due to necessary amputation of the third toe, the middle phalange of which was nearly completely destroyed. The findings were as follows: the epidermis became somewhat thinner, incorrespondance with the rather flattened papillae of the corium; in the stratum reticulare perivascular infiltrations were noticed; extensive areas of the granulation tissue, situated more deeply, were coming close to the bones and penetrated into them. The cells aggregated in clusters formed separate nodules. They consisted mainly of lymphoid cells which changed their character in the deeper layers between the laminae of the bones, due to the adding of the giant cells, which were situated beyond the nodules in the fibrous scar tissue.

In the areas where the granulation tissue penetrated into the bone, the periosteum lost its structure, and an overgrowth of the bone tissue took place. The clusters of red blood cells were not rarely seen in the central parts of the nodules. Histiocytes, fibroblasts and sometimes leucocytes could be observed in the vicinity of the areas of necrosis and near them there were situated larger cells of the osteoyd tissue (stained with hematoxylin-eosin). In the lumen of the vessels the aggregation of the leucocytes could be seen. The bone marrow was mostly changed, the same was with the bone tissue. If Van-Gieson stain was used, the predominance of the fibrous tissue was very clearly seen. Not only the destruction of bone tissue was noticed but also its new growth, characterized by the close resemblance of the tissue with the cartilage and the imperfect formation of the bone laminae.

The mycelium of the fungus was well stained by the method of Gram-Bogolepov (and even by the usual staining with hematoxylin-eosin). There were of especial interest not only great accumulations of mycelium and its presence in the areas of lesions but also the reaction of tissues, to the invasion of pathogenic fungi. The connective tissue with few cells was developed in the areas of lesions, but the outlines of the nuclei of these cells were not changed. This

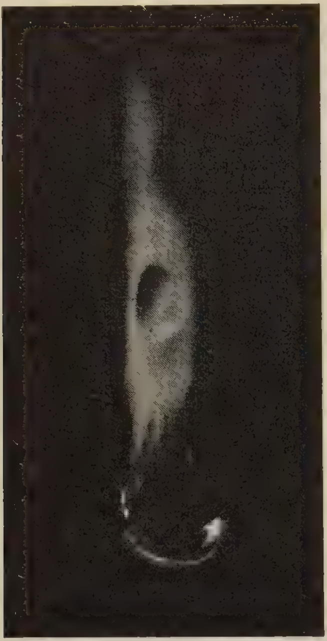


Fig. 2-a. The culture of *Trichophyton crateriforme*, obtained on the Sabouraud media, after inoculation of the discharge from the fistula on the plantar side of 3rd toe.

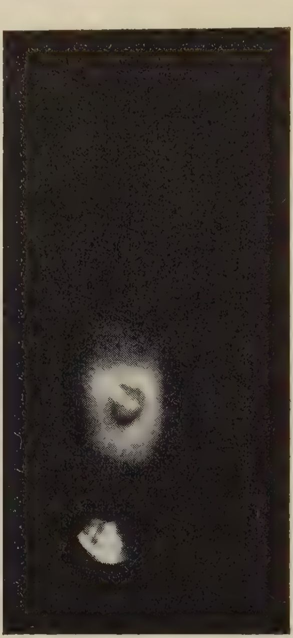


Fig. 2-b. The culture obtained after inoculation of the discharge of fistula in the region of right lymphadenitis inguinalis.

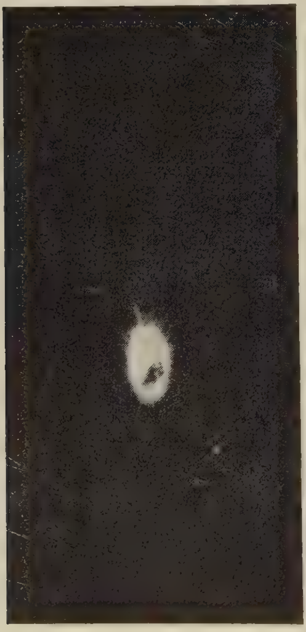


Fig. 2-c

Fig 2-d

Fig. 2-e

Fig. 2-f

Fig. 2-g

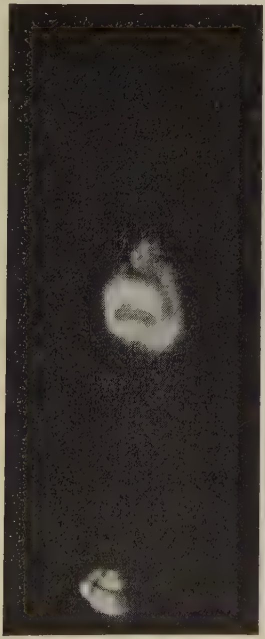


Fig. 2-h

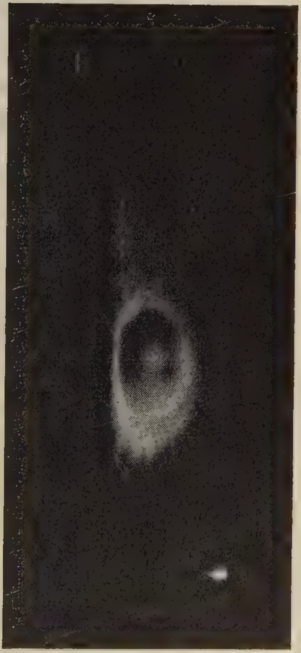


Fig. 2-i

Fig. 2-c, d, e, f, g, h, i- the cultures from the lesions of glabrous skin, scalp, hairs and nails.

tissue was loose and unevenly stained, lying near the bone tissue in some places protruding from it, and in other places it was in the state of necrobiosis. The massive areas of necrosis with abundant mycelial filaments were located on the level of subcutaneous tissue near the skin. Especially much mycelium was in the area of destruction, connected with the surface of the skin. This allowed to find the fungi without any difficulty before the operation in the

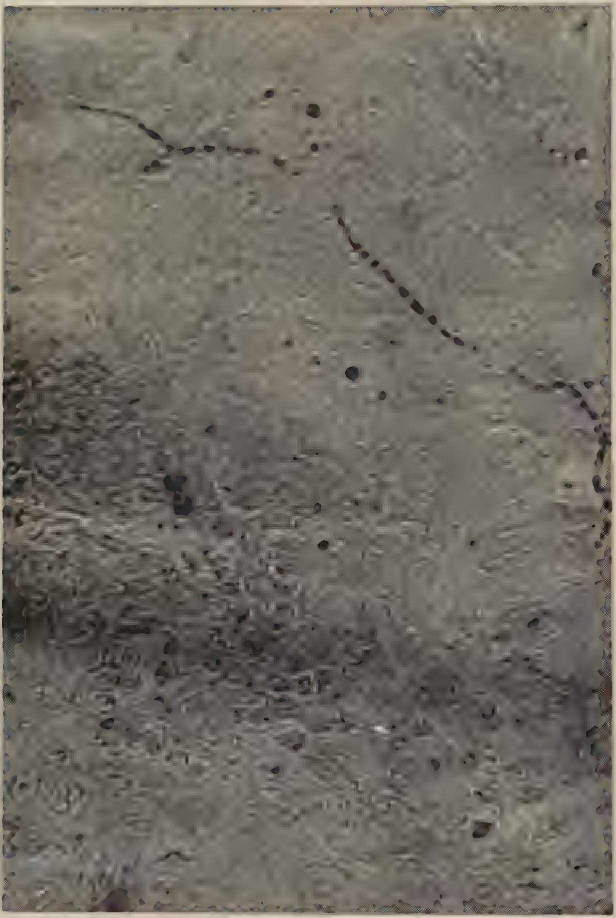


Fig. 3. Photomicrogram of a histopathologic preparation of the middle phalange of the third toe of the right foot (stained with hematoxylin-eosin, dry system, great magnification): amidst detritus, separate cells and fibrous connective tissue are seen; the filaments of mycelium having a characteristic structure (the barrel-like segments, which are interchanged with good-stained segments).

pathological material discharged from the fistula and to obtain many times the culture of *Trichophyton crateriforme.* Areas of lesions of soft tissues located in the immediate vicinity of the changed bones were of special interest. They had the character

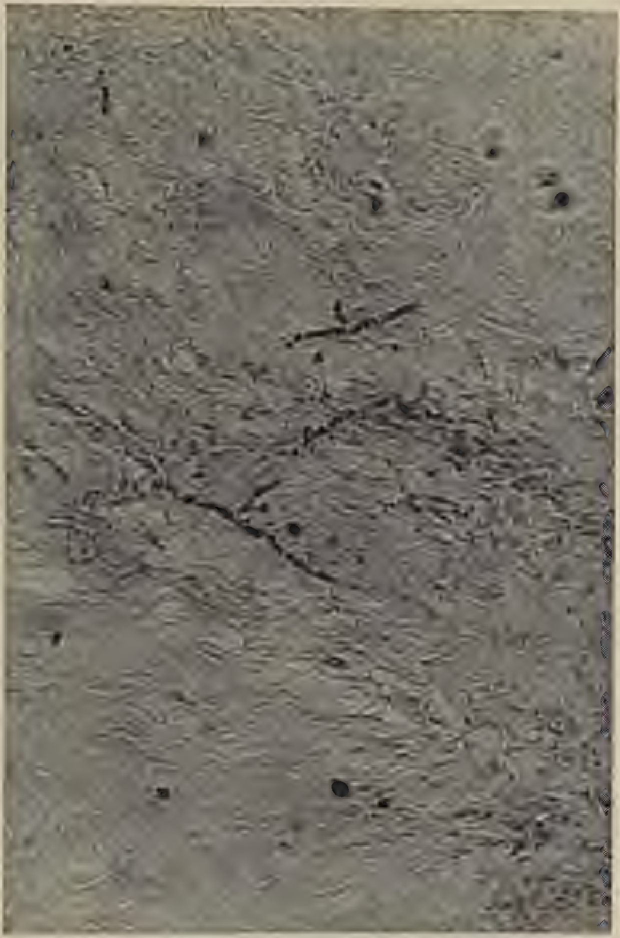


Fig. 4. The same elements of fungus amidst mainly fibrous connective tissue, long filaments, dichotomously branching, the clusters of spores.

of granulomes with many giant cells, sometimes enclosing filaments and fragments of mycelia. Sometimes the giant cells, when phagocytozing the fungi, lost the bright colour of their nuclei. Filaments of the fungi were especially clearly seen. Rarely it was vice versa;

the filaments of the fungus, its fragments were badly stained, leaving only slight outlines in the protoplasma of the phagocyte. It was in these areas of accumulation of fungal elements that the signs of destruction, necrosis, necrobiosis, and resorption of elements of bone tissue were especially clearly seen. As far as the lesion of the bone itself is concerned, mention should be made of the giant cells, which not rarely contained living, brightly stained mycelium, generally septate. More often, however, so in this case, the cell itself, and sometimes even its outlines, perhaps due to the strong

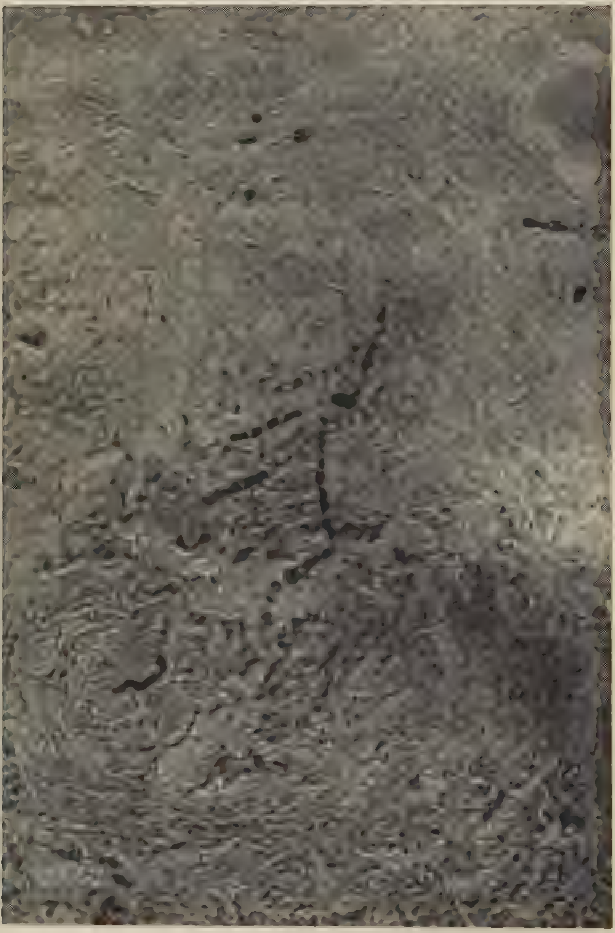


Fig. 5. same as fig. 4.

action of trichophyton, lost the capacity to be stained. In the swollen fibrous tissue, which often substituted bone tissue, among its separated fibres, sometimes rather long filaments of mycelium were also seen. The capacity of *Trichophyton endothrix crateriforme* to propagate and to freely grow through different organs and tissues, destroying in some places barriers of giant cells, is of great interest. Comparatively anaerobic conditions don't hinder its growth and development in the fibrous tissue and in the bone tissue.

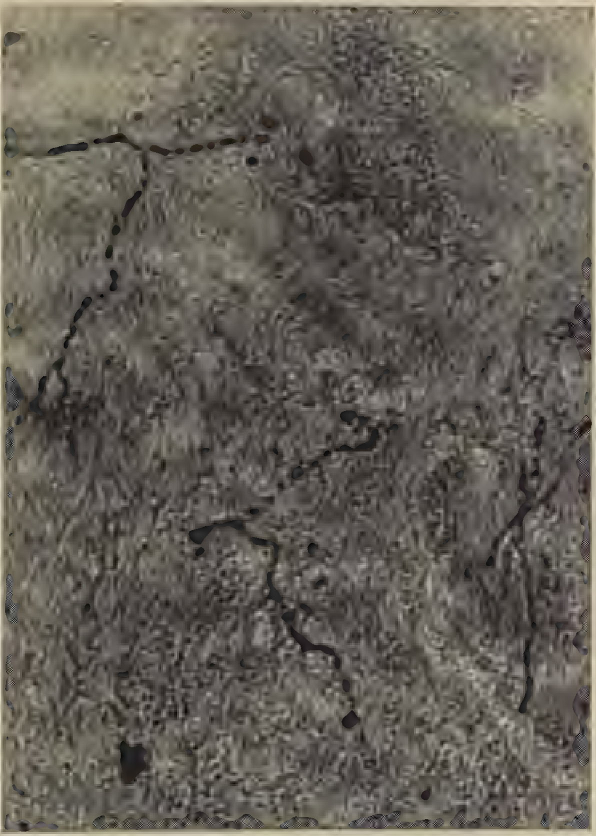


Fig. 6. same as fig. 4.

On the photomicrograms they are seen amidst detritus and the remaining islands of cell elements and connective tissue. The unevenly stained segments are very well seen; diffusely stained segments are interchanging with swollen barrel-like segments, with unstained center. Separate, intensively stained spores may be seen. Amidst principally fibrous tissue which mostly has retained its structural peculiarities the same elements of the fungus are seen; some filaments have a great diameter and are a bit swollen at the ends. (Fig. 5, 6).

On the photomicrogram one can see good growth and development of the fungus. The mycelium attains great length and begins to branch dichotomously. At the ends of mycelium and be side them spores can be found, which are well stained. Some of them have a clear halo around them, others are aggregated in clusters containing several spores (up to five). (Fig. 6).

Discussion

The chronic process — the lesion of three toes of the type of osteomyelitis, which has developed on the place of a stab wound, had a trichophytid etiology. Phalangitis was characterized by a productive degenerative process in the bone, by the lack of expressed suppuration, by a partial substitution of the bone by fibrous connective tissue, by new growth of bone tissue in some places, and by resorption of cartilage in other places.

The causes for the development of areas of trichophytia of bones were: a chronic trichophytia of long duration in the family and in the patient and a stab wound made with a pitchfork. The etiologic significance of *Trichophyton crateriforme* is confirmed by: 1) the finding of the elements of fungus in deep areas of chronic inflammation; 2) their capacity of growth and development; 3) good staining even during the phagocytosis; 4) a considerable reaction of the tissue to the invasion of the pathogenic fungus; 5) the presence of fungus in the products of destruction, and in pus discharged from the fistulae; 6) positive cultures of *Trichophyton crateriforme* from the basal trichophytid bone lesion as well as from all the other areas of trichophytia of the patient (and her brother).

In this respect, the discovery of mycelial filaments and the positive culture of *Trichophyton crateriforme* in the content of suppurated regional (right femoral) lymphatic node has also some importance.

Bone lesions caused by trichophytons were for the first time described by a Moscow mycologist, professor CHERNOGUBOV who published in 1926 (together with A. Y. PELEVIN) a description of the trichophytid lesion of sternum and of the 4-10 right ribs in a patient, who has suffered, as well as two other members of her family, from chronic trichophytia of adults. She showed leftsided skoliosis, tenderness of vertebrae during the palpation from the first lumbar until the fifth thoracic vertebrae, existence of fistulae connecting deep lesions with superficial integument, mycelial filaments in the discharge of these fistulae. Cultures of *Trichophyton* *violaceum* were obtained from the discharge of fistulae and from other pathological products of the patient and the lesions in her brothers yielded the same fungus. The peculiarities of X-ray pictures in many respects supported also the results of our investigations: the rarefaction of the bones, the defects on the edges of the ribs, the sloping grooves, even the disjunction of the ends of the rib (9). Areas of clearly expressed proliferative process, the development of bone tissue, the approximation of the ends of adjoining ribs were also seen.

Summary and Conclusion

The histopathologic method is the most important among the methods of complex mycological examination of deep lesions of different organs caused by dermophytes, common in our practice. It is this method in comparison with cultural, microscopic, experimental and other investigative methods that gives us the possibility to make a conclusive decision about the etiology of deep lesions of individuals suffering from mycoses. Systematic, complex examination of the patient, the permanent part of which was the histopathologic method gave us the possibility, alongside with other rare findings to obtain a conclusive material in favour of the existence of trichophytia of bones. This way we could greatly develope our knowledge of the nature of the process of trichophytia.

1. The causative agent *Trichophyton crateriforme* was found after inoculating pathologic material from the trichophytid lesions of the bones and other parts of the body.

2. Trichophytia of bones developed after a trauma in the patient suffering from chronic trichophytia adultorum.

3. The existence of secondary specific lymphadenitis was proved.

4. X-ray examination revealed: complete destruction of middle phalange and partial destruction of the basal phalange (of the third toe), osteoporosis of bones of the foot — real atrophy, in some places — endostal deposits.

5. Histopathologic examination showed: a) chronic process of inflammation — granulation tissue, its penetration into the bones, the periosteum losing its outlines, the existence of lymph nodes with giant cells, and sometimes a necrosis in the center, big osteoid cells, histocytes, fibroblasts, and white blood cells; a clustering of the red blood cells; b) destruction of bone tissue and growth of the granulation tissue in its place, change of the structure of the bone marrow, the development of osteoid tissue.

6. Further evidence was established by the peculiarities of the pathogenic fungus in the areas of lesions, the abundance of mycotic filaments both in the area of lesion and in the discharge of fistulae, the growth and development of vigorous, long, branching, well stained mycelium, the peculiar structure of different tissues (including roughly fibrous, fibrous and bone tissue).

RIVISTA CRITICA DELLA LETTERATURA MICOLOGICA MEDICA IN ITALIA TRA IL 1946 ED IL 1956

Prof. P. Pinetti

(*Direttore della Clinica Dermatologica e del Centro di Micologia dell'Università di Cagliari*)

(17.II.1959)

I. DERMATOMICOSI E DERMATOFITI

Le tigne e le epidermomicosi sono particolarmente frequenti in Italia e pur essendo numericamente diminuite nei confronti del passato, costituiscono ancor oggi un problema sociale di particolare importanza (27.28.57) soprattutto per la notevole diffusione e per l'andamento epidemico che esse assumono in alcune regioni (3.10. 24.61).

In base ad un'accurata indagine statistica riguardante il decennio 1945—54 Riggio (56) ha tracciato un quadro sufficientemente esatto della situazione epidemiologica delle tigne e delle epidermomicosi nelle regioni dell'Italia centro-meridionale. I dati conclusivi di questo studio sono condensati nella seguente tabella.

Tabella I.

Frequenza delle dermatomicosi in Italia dal 1945 *al* 1954 *ed indici decennali di morbilità rispetto alla popolazione assoluta regionale* (Riggio)

REGIONI	Tigne					Epidermomicosi
	Num.casi	Indice di morbilità %	Forma clinica			Indice di morbilità %
			T.tric.	T.micros.	T.fav.	
Sardegna . . .	4238	0.347	93 %	1 %	6 %	—
Puglia	7547	0.242	80	0.5	19.5	0.019
Calabria . . .	4190	0.200	98	1.5	0.5	0.072
Sicilia	8730	0.199	50	35.0	15.0	0.083
Lazio.	3246	0.100	90	10.0	—	0.119
Campania . .	2941	0.069	70	20.0	10.0	0.040
Lucania . . .	309	0.050	75	25.7	0.3	0.020
Abruzzo . . .	461	0.027	90	—	10.0	0.119
Umbria . . .	189	0.020	75	15.0	10.0	—
Toscana . . .	629	0.020	98	—	2.0	0.037
Marche	161	0.012	80	20.0	—	0.004

Nelle regioni del Nord-Italia le tigne compaiono con una frequenza piuttosto limitata (1).

Predominano nella quasi totalità delle regioni centro-meridionali della penisola le tigne tricofitiche che in media costituiscono da sole il 70—90 % delle micosi del cuoio capelluto. Le microsporie sono particolarmente frequenti in Sicilia, Campania e Lucania e sembrano farsi in questi ultimi anni progressivamente sempre più frequenti. SBERNA (59) ha descritto nel 1951 a Firenze una vera epidemia di tigna microsporica. Osservazioni personali inedite portano a documentare anche in Sardegna un marcato aumento delle microsporie, probabilmente legato all'ampliarsi sempre maggiore dei rapporti con la Sicilia.

La tigna favosa si mantiene invece entro limiti di scarsa diffusione, con qualche focolaio di maggior frequenza in Puglia (20 % delle tigne) ed in alcune zone del nord.

La flora dermatomicetica Italiana, che in passato era stata oggetto di attento studio si può dire in tutte le regioni della penisola, è stata ripresa in sistematico esame in quest'ultimo decennio solo in poche zone. A Pavia ABBAMONTE (1) trova *Trichophyton* del gruppo *crateriforme* nel 54,5 % dei casi, *T. rosaceum* nel 9,6 %, *T. schönleini* nel 9,3 %, *T. faviforme album* nel 4,8 %, *T. mentagrophytes* nel 25,9 % e *T. violaceum* nel 3,3 %. Dei dermatomiceti isolati a Pescara da DONATELLI (22) il 5,1 % è costituito da *Trichophyton* del gruppo *crateriforme*, il 5,8 % da *T. rosaceum*, il 10,3 % da *T. schönleini*, il 3,8 da *T. mentagrophytes* ed il 75 % da *T. violaceum*. Gli isolamenti effettuati a Roma da D'ARCA (19.20) si riferiscono per l' 1,8 % a *T. crateriforme*, per il 5,4 % a *T. schönleini*, per l' 1,8 % a *T. faviforme ocraceum*, per il 7,2 % a *T. mentagrophytes*, per il 43,6 a *T. violaceum* e per il 40 % a *Microsporum lanosum* e *M. audouini*.

A Cagliari SPADA & ZONCHELLO (71) hanno trovato una scarsa varietà di specie dermatofitiche che si riducono a *T. schönleini*, *T. crateriforme* (27,7 %) *T. megnini* (8 %), *T. mentagrophytes* (1,8 %) e *T. violaceum* (62,5 %).

Da un quadro complessivo della flora dermatomicetica italiana tracciato da PINETTI (51) risulta che *T. violaceum* costituisce la specie tricofitica assolutamente predominante nella gran parte della penisola, fatta eccezione per le regioni del nord e della Sicilia dove prevalgono o sono particolarmente frequenti *T. mentagrophytes*, *T. crateriforme* e *M. canis*. In generale l'ordine di frequenza delle diverse specie tricofitiche può essere stabilito come segue: 1) *T. violaceum*, 2) *T. crateriforme*, 3) *T. mentagrophytes*, 4) *T. schönleini*, 5) *T. megnini*, 6) *T. rubrum* e 7) *T. verrucosum* (= *T. discoides*, *T. album*).

Tra i *Microsporum* frequente il *M. canis* e piuttosto raro *M. audouini*.

L'esistenza e l'importanza delle infezioni dermatomicetiche miste è stata studiata da SPADA (68) su di un piano generale ed a proposito di alcune osservazioni personali.

La morfologia clinica delle dermatomicosi è stata oggetto di interessanti contributi. BESSONE (7, 8, 9), MARIOTTI (34), PAIS (39) e LATTE & PIREDDA (31) hanno descritto casi di tricofizia e di microsporia del sopraciglio e blefarociliare; CARLI (14) e ROMANO (59) aspetti anomali di favo delle parti glabre. BOSCO (12), MONTANARO (36) e SPADA (70) hanno affrontato il discusso problema delle tigne del cuoio capelluto in soggetti adulti ponendo in rilievo alcune condizioni disendocrine che potrebbero facilitare l'attecchimento dei miceti o favorire la persistenza di infezioni contratte nell'infanzia.

LISSIA (33) ha descritto un caso di tigna in un soggetto portatore di aplasia moniliforme del capillizio; MUSUMECI (37) studiando un interessante caso di affezione granulomatosa sostenuta da *T. violaceum,* ha sottolineato i rapporti clinici e patogenetici che intercorrono tra granuloma tricofitico e follicolitis suffodiens et abscedens di Hoffmann. LEONE (32) ha richiamato l'attenzione sulla frequenza di reperti micotici nelle onicogrifosi.

Alcuni problemi immunitari sono stati discussi a proposito delle tricofitidi e microsporidi (6, 25) e di alcuni quadri micotici trofoallergici (35). Mentre SERRI (64) non ha trovato nel sangue di soggetti colpiti da dermatosi di tipo infiammatorio alcuna attività fungistatica o fungicida, PIREDDA (55) riscontra nel liquido di bolla di soggetti affetti da dermatomicosi una concentrazione in anticorpi specifici antitricofitinici più elevata che nel siero.

BERGAMASCO (5) ha elaborato un nuovo metodo di preparazione e purificazione della tricofitina diagnostica basato sull'estrazione fenolica.

Della terapia delle tigne e delle dermatomicosi si sono occupat) diversi studiosi (2, 11, 17, 21, 30, 44, 58, 60, 72, 74). SPADA (69-ha richiamato l'attenzione su di un particolare fenomeno di ricresci cita precoce dei capelli dopo roentgendepilazione nelle zone di cuoio capelluto precedentemente interessate dalle chiazze tignose.

Per la ricerca dei miceti nei materiali cutanei TAGLIAVINI (73) ha elaborato un metodo che modifica utilmente quello proposto da KLINGMANN & MESCON, rendendone più agevole e più rapida l'esecuzione.

Particolarmente numerosi ed interessanti sono i contributi portati dagli studiosi italiani nel campo della biologia dei dermatomiceti. Il pH del substrato e la sua influenza sullo sviluppo micetico a seconda delle diverse specie è stato studiato da ORRU' (38). PINETTI (40, 41) ha precisato con ricerche sistematiche le capacità di assimilare le varie fonti idrocarbonate ed azotate e le attività glicolitiche e proteolitiche di diverse specie di dermatomiceti. Sono state inoltre precisate le influenze che alcuni composti organici (4, 13, 62), alcune vitamine (29) ed alcuni antibiotici (63, 65) esercitano in senso favorente od ostacolante sullo sviluppo in vitro dei dermatomiceti.

La morfologia e la biologia di *Trichophyton violaceum* sono state particolarmente studiate dalla Scuola Micologica di Cagliari.

Pinetti ha indagato nei suoi vari aspetti la pigmentogenesi di questo micete (42, 43), la sua variabilità morfologica spontanea ed indotta (46) e le sue attività proteolitiche in rapporto con le variazioni della macromorfologia delle culture (52).

Ciferri (15) ha dimostrato l'esistenza in alcuni dermatomiceti della capacità di produrre sostanze ad attività antibiotica, Pinetti & Spada (53) riscontrano nel filtrato di coltura di alcuni dermatofiti un'attività antidotica che si manifesta riducendo il potere emolitico della tossina stafilococcica e Pirastu (54) un attività fitotossica che si esplica ostacolando lo sviluppo delle radici di lupino.

Oggetto di particolare studio sono state le reciproche influenze di ordine antagonistico che si determinano tra miceti di diversa specie. Spada (66, 67) ha documentato l'esistenza e studiato le modalità di estrinsecazione di alcune influenze inibitorie che si manifestano tra alcuni dermatofiti del genere *Trichophyton*, *Microsporum* ed *Epidermophyton* in diverse condizioni sperimentali.

In una serie di lavori è stato largamente studiato il fenomeno di blocco della reazione tirosina/tirosinasi descritto da Robinson e coll. nelle colture di *Microsporum audouini*. Confermata l'esistenza del fenomeno in una vasta serie di altre specie dermatofitiche, Pinetti ed i suoi collaboratori hanno dimostrato che esso non è dovuto, come era stato da altri prospettato, ad un blocco della attività enzimatica tirosinasica, ma bensì alla scomparsa o alla diminuzione nel substrato della tirosina che viene utilizzata in misura diversa dai vari dermatofiti. (45, 47, 48, 49, 50).

Giardini & Serri (26) hanno studiato la patogenicità dei dermatofiti per i tessuti oculari giungendo a conclusioni negative e Farina (23) ha documentato sperimentalmente l'istaurarsi di una iperrecettività dei tessuti cutanei in seguito a piccole ripetute infezioni con materiale dermatofitico.

Ciferri & Redaelli non sono riusciti ad ottenere formazioni ascofore o picnidiali coltivando alcuni dermatofiti su substrati naturali (16).

Bibliografia

1. Abbamonte, V.: Indagini sui miceti causa di dermatomicosi nella provincia di Pavia. Boll. Soc. Med. Chir. Pavia. **46**, **313**, **1952**.
2. Agostini, A.: Osservazioni sulla moderna terapia delle epidermomicosi. Minerva Derm. Atti SIDES. **30**, 95, 1955.
3. Agostini, A.: Osservazioni sulla frequenza e sugli attuali aspetti clinici delle epidermomicosi. Arch. Ital. Derm. **26**, 409, 1954.
4. Argenziano, G. & d'Atri, G.: Sull'influenza della lecitina sullo sviluppo colturale di alcuni miceti delle tigne. Ann. Ital. Derm. **6**, 58, 1951.
5. Bergamasco, A.: Metodo di preparazione della tricofitina diagnostica median te l'estrazione fenolica. Arch. Ital. Derm. **25**, 87, 1952.
6. Bellafiore, V. & Palmintieri, G.: Lichen tricofitico in corso di trattamento penicillinico di tigna profonda suppurativa. Dermatologia. **6**, 35, 1955.
7. Bessone, L.: Due casi di microsporia del sopraciglio. Aggiorn. pediatr. **2**, 11, 1951.

8. Bessone, L.: Due casi di tricofizia del sopraciglio. Dermosifilografo. **25,** 312, 1955.
9. Bessone, L.: Altri due casi di microsporia del sopraciglio. Minerva Derm. **30,** 172, 1955.
10. Bessone, L.: Su di una epidemia di tigna tricofitica in Genova. Aggiorn. Pediatr. **6,** 301, 1955.
11. Boiti, D.: Note di tecnica sulla roentgendepilazione. Rass. Derm. Sif. **5,** 215, 1952.
12. Bosco, I.: Considerazioni su di un caso di tricofizia ultraresistente della cute e degli annessi in donna adulta. Rass. Derm. Sif. **6,** 49, 1953.
13. Carbonera, P.: Azione fungistatica dell'acido undecilenico su alcuni ceppi fungini. Riv. Ist. Sier. Ital. **26,** 43, 1951.
14. Carli, G.: Favo epidermico dello scroto ed eritematosquamoso della cute glabra. Arch. Ital. Derm. **22,** 49, 1949.
15. Ciferri, R.: Attività antibiotica di alcuni dermatofiti. Mycopathologia. **4,** 219, 1947.
16. Ciferri, R. & Redaelli, P.: Mancata formazione di forme ascofore e conservazione di colture di funghi patogeni in substrati naturali. Mycopathologia. **4,** 131, 1948.
17. Cofano, A. R.: La terapia delle tigne. Dermatologia. **4,** 110, 1953.
18. Cottini, G. B.: Il problema delle parassitosi cutanee nella provincia di Catania come risulta dai dati della Clinica Dermosifilopatica. Atti Soc. Ital. Derm. **4,** 244, 1941.
19. d'Arca, S.: Incidenza epidemiologica dei tricofiti nella provincia di Roma. Nota II°. Nuovi Ann. Igiene Microb. **7,** 465, 1956.
20. d'Arca, S.: Incidenza epidemiologica dei tricofiti nella provincia di Roma. Nota III°. Nuovi Ann. Igiene Microb. **7,** 469, 1956.
21. d'Atri, G.: L'acido undecilenico nella cura delle epidermomicosi. Ann. Ital. Derm. **5,** 264, 1950.
22. Donatelli, G.: Le tigne nella provincia di Pescara. Atti II° Conv. Med. Osp. Abruzzese. 1947.
23. Farina, L.: Ricerche sul decorso delle infezioni sperimentali da dermatofiti in rapporto ad un eventuale stato di iperrecettività provocata. Mycopathologia. **4,** 162, 1947.
24. Gardenghi, C.: Rilievi statistici e provvedimenti profilattici nel campo delle tigne flogistiche del rurale. Rass. Med. Industr. **22,** 346, 1953.
25. Giardini, A. & Farina, L.: Microsporide congiuntivale nel corso di microsporia infiammatoria cutanea. Ann. Oftalm. **72,** 691, 1946.
26. Giardini, A. & Serri, F.: Potere patogeno dei dermatomiceti sull'occhio. Mycopathologia. **4,** 172, 1948.
27. Giardino, G.: Proposte per il depistage delle tigne. Dermatologia. **2,** 228, 1951.
28. Giardino, G.: Piano di lotta contro le micosi. Dermatologia. **3,** 240, 1952.
29. Grixoni, F.: Rilievi sull'influenza delle vitamine sintetiche sullo sviluppo colturale di determinati ifomiceti patogeni per l'uomo. Boll. Soc. Ital. Biol. Sper. **24,** 638, 1948.
30. Grixoni, F.: Quindici anni di roentgenterapia delle tigne nella Clinica Dermosifilopatica dell'Università di Sassari. Minerva Derm. Atti SIDES. **30,** 687, 1955.
31. Latte, B. & Piredda, A.: Localizzazioni blefarociliare di epidermofizia diffusa da Trichophyton gypseum asteroides. Boll. Ocul. **33,** 761, 1954.
32. Leone, R.: Sulle onicogrifosi micosiche. Minerva Derm. **19,** 281, 1954.
33. Lissia, G.: Tigna tricofitica ed aplasia moniliforme. Dermatologia. **7,** 101, 1956.
34. Mariotti, L.: Un caso di tricofizia del sopraciglio. Ann. Oftalm. **76,** 38, 1951.
35. Molinari, R.: Di un particolare quadro trofo-allergico secondario ad epidermomicosi da Trichophyton schönleini. Giorn. Ital. Derm. **97,** 69, 1956.
36. Montanaro, E.: Tigna torpida tonsurante insorta in soggetto adulto affetto da nanismo. Arch. Ital. Derm. **21,** 364, 1948.
37. Musumeci, V.: Studio comparativo tra granuloma tricofitico di Majocchi e

la perifollicolitis capitis abscedens e suffodiens di Hoffmann. (A proposito di un caso clinico). Minerva Derm. **31**, 235, 1956.

38. Orru', A.: Studi sulla biologia dei dermatomiceti. Influenza del pH sullo sviluppo colturale in terreni liquidi. Rass. Med. Sarda. Suppl. **50**, 74, 1948.
39. Pais, L.: Le tricofizie del sopraciglio. Antologica. 1946.
40. Pinetti, P.: Studi sulla biologia dei dermatomiceti. Nota I⁰. Capacità di assimilazione dell'azoto ed attività proteolitiche di alcuni dermatomiceti. Rass. Med. Sarda. **47**, 54, 1945.
41. Pinetti, P.: Studi sulla biologia dei dermatomiceti. Nota II⁰. Capacità di assimilazione degli idrati di carbonio ed attività fermentative glicolitiche di alcuni dermatomiceti. Mycopathologia. **4**, 222, 1948.
42. Pinetti, P.: Studi sulla pigmentogenesi di Trichophyton violaceum. Nota I⁰. Influenza esercitata sulla pigmentogenesi del Trichophyton violaceum da fattori chimici e fisici agenti sul substrato colturale. Rass. Med. Sarda. **51**, 179, 1949.
43. Pinetti, P.: Studi sulla pigmentogenesi di Trichophyton violaceum. Nota II⁰. Estrazione e purificazione del pigmento. Rass. Med. Sarda. **51**, 196, 1949.
44. Pinetti, P.: Impiego della penicillina nel trattamento del kerion tricofitico. Terap. antib. e Chemiot. 1953.
45. Pinetti, P.: Influenze inibitrici esercitate da alcuni dermatomiceti sulla reazione tirosina/tirosinasi. Minerva Derm. Atti SIDES. **30**, 717, 1955.
46. Pinetti, P.: Studi sulla variabilità di Trichophyton violaceum. Aspetti macromorfologici. Nota I⁰. Riv. Ist. Sier. Ital. **31**, 441, 1956.
47. Pinetti, P.: Su di una particolare influenza esercitata da alcune specie dermatomicetiche sul complesso tirosina/tirosinasi. Boll. Soc. Biol. Sper. **32**, 1448, 1956.
48. Pinetti, P.: Influenze dei sali di rame nella genesi dell'inibizione esercitata da alcuni dermatomiceti sulla reazione tirosina/tirosinasi. Boll. Soc. Ital. Biol. Sper. **32**, 1449, 1956.
49. Pinetti, P.: Su di una particolare attività esercitata sul complesso tirosina/tirosinasi da alcune specie dermatomicetiche. Acta Vitamin. **5**, 229, 1956.
50. Pinetti, P.: Su di una semplice metodica per l'allestimento di sospensioni omogenee da coltura di dermatofiti. Boll. Soc. Ital. Biol. Sperim. **32**, 1372, 1956.
51. Pinetti, P.: La flora dermatomicetica d'Italia. Rass. Med. Sarda. **59**, 47, 1957.
52. Pinetti, P. & Spada, C.: L'attività proteolitica di Trichophyton violaceum ed il suo comportamento in rapporto con la variabilità del micete sui terreni artificiale di coltura. Boll. Soc. Ital. Biol. Sper. **31**, 1031, 1955.
53. Pinetti, P. & Spada, C.: Sull'attività antidotica di filtrati di coltura di dermatomiceti. Nota I⁰. Azione sulla tossina stafilococcica. Minerva Dermat. Atti SIDES. **31**, 536, 1956.
54. Pirastu, A.: Sulla presenza nei filtrati di colture di dermatomiceti di sostanze ad azione fitotossica. Minerva Derm. Atti SIDES. **30**, 37, 1955.
55. Piredda, A.: Della produzione di anticorpi antiifomicetici da parte della cute umana. Giorn. Ital. Derm. **96**, 527, 1955.
56. Riggio, T.: Epidemiologia e profilassi delle tigne e delle epidermomicosi nell'Italia centro-meridionale ed insulare durante il decennio 1945—54. Atti VI⁰ Conv. Ass. Ispett. Dermosif. Roma. 1955.
57. Rizzi, V. & Magliari, G.: Il problema delle tigne nella provincia di Lecce. Arch. Ital. Derm. **27**, 457, 1955.
58. Rizzi, V. & Potenza, V.: Sulla cura dei tignosi col metodo combinato dell'acetato di tallio e dei raggi Röntgen. Minerva Derm. Atti SIDES. **30**, 687, 1955.
59. Romano, S.: Favo generalizzato cronico in soggetto anergico. Ann. Ital. Derm. **4**, 33, 1949.
60. Sabatini, G.: Gli acidi grassi insaturi nel trattamento delle micosi cutanee. Ann. Med. Nav. e Trop. **56**, 368, 1951.
61. Sberna, P.: Su di un'epidemia di tigna microsporica in Firenze. Rass. Derm. Sif. **4**, 355, 1951.

62. Serri, F.: Azione dell'acido paraminobenzoico sulla crescita di alcuni dermatomiceti. Acta Vitam. **3,** 73, 1948.
63. Serri, F.: Azione in vitro di alcuni antibiotici: penicillina, streptomicina, tirotricina, furazina sullo sviluppo dei dermatomiceti. Il Farmaco. **3,** 535, 1948.
64. Serri, F.: Ricerche sul potere fungistatico e fungicida del sangue nelle dermatomicosi. Mycopathologia. **4,** 139, 1948.
65. Serri, F.: Pouvoir antifongique de la polimixine (aerosporine) sur huit souches de dermatophytes. C. R. Soc. Biol. **143,** 362, 1949.
66. Spada, C.: Influenze reciproche esercitate sulla loro crescita da diversi tipi di dermatomiceti. Rass. Med. Sarda. Suppl. **51,** 120, 1949.
67. Spada, C.: Ulteriori ricerche sulle influenze reciproche esercitate sulla loro crescita da diversi stipiti di dermatomiceti. Boll. Soc. Ital. Biol. Sper. **31,** 1028, 1955.
68. Spada, C.: Le infezioni dermatomicetiche miste. Rass. Med. Sarda. **57,** 121, 1955.
69. Spada, C.: Ricrescita precoce a focolaio dei capelli in soggetti sottoposti a roentgendepilazione per affezioni micotiche del cuoio capelluto Rass. Med. Sarda. Suppl. **51,** 115, 1949.
70. Spada, C.: Considerazioni su tre casi di tigna tricofitica del cuoio capelluto in donne puberi. Rass. Med. Sarda. **54,** 159, 1952.
71. Spada, C. & Zonchello, S. A.: Studio sulle variazioni determinatesi nella flora dermatomicetica di Cagliari nell'ultimo quinquennio. Rass. Med. Sarda. **54,** 149, 1952.
72. Sprecher, A.: L'acido oleico come depilante e la sua applicazione nella cura delle tigne. Arch. Ital. Derm. **25,** 208, 1952.
73. Tagliavini, R.: Proposta di alcune modifiche al metodo di Klingmann-Mescon per la ricerca dei miceti. Arch. Ital. Derm. **28,** 160, 1956.
74. Trimigliozzi, G.: Il trattamento locale delle tigne senza depilazione. Rass. Clin. Scient. **26,** 149, 1950.

II. CANDIDA ALBICANS ED ALTRI MICETI LIEVITIFORMI E MICOSI DA ESSI DETERMINATE

Cazzola e coll. (13, 14, 15, 16, 17) hanno studiato la flora ifomicetica e saccaromicetica della vagina della donna sana e precisato le variazioni qualitative e quantitative che essa presenta nelle diverse età della vita. Colonnello (19) ha potuto precisare il significato biologico e patologico di alcuni miceti lievitiformi dell'intestino umano.

Interessanti azioni di interferenza di *Candida* sul potere antibatterico di alcuni antibiotici sono state descritte e studiate da Caputi (8, 9), Masoni & Papinutto (46) e di Bella (23).

Magni (42) studiando le curve di sopravvivenza alla roentgenirradiazione riesce a documentare un diverso comportamento della ploidia in varie specie di lieviti asporigeni precisando che *Torulopsis pulcherrima* e *T. neoformans* sono permanentemente aploidi.

Verona & Florenzano (58) dimostrano che le strutture ameboidi delle blastospore di *Candida pulcherrima* sono probabilmente stadi che precedono la formazione di pseudomicelio.

Cipriani (18) sostiene che la natura del substrato e l'età della coltura non influenzano la positività della reazione di Dold in *Geotrichum candidum*. Castello (12) rileva che *Candida albicans* è sensibilmente meno resistente dei batteri all'azione degli ultrasuoni. Zini (62) ha potuto dimostrare che l'inoculazione di *Candida* *albicans* nella membrana corio-allantoidea dell'embrione di pollo può servire per accertarne la patogenicità, GARGANI (30) che l'inoculazione intradermica del coniglio costituisce un mezzo valido per la valutazione della virulenza delle varie specie di *Candida*.

FORNI e coll. (29) documentano la diversa costituzione enzimatica delle varie specie di *Candida* e la diversa modalità con cui esse determinano la scissione degli aminoacidi e ZAMBOTTI & MORET (60) precisano la capacità di sintesi delle proteine da parte di *Oospora lactis*. FERRARIS (25) ha potuto riscontrare in *Oospora virescens* l'esistenza di un'attività antibiotica verso diverse specie di miceti. CAPUTI ha studiato la dissociazione microbica che il lisozima induce in *Mycothorula albicans* (10) ed i fenomeni di antagonismo che possono esser messi in evidenza tra questo micete e stafilococco (11). MAGNI (43) ha indagato sul significato dell'inibizione reciproca che si determina tra colonie di lieviti asporigeni nei riguardi della formazione di pseudomicelio. GIGLI (33, 34) è riuscito ad ottenere una resistenza indotta in *Candida albicans* verso alcune sostanze coloranti.

Le micosi sperimentali da *Candida albicans* sono state studiate da FORNI (27, 28) nel topo albino. LANZARA (37, 38) ha ottenuto focolai granulomatosi a diversa evoluzione mediante inoculazione diretta del micete nella lingua e nel fegato ed URSO (56) una broncopolmonite negli animali mediante inalazione.

In una serie di interessanti lavori PIANTONI e coll. (48, 49, 50) e BARBAZZA (4) hanno studiato il quadro istopatologico delle lesioni che conseguono alla inoculazione per via endovenosa, endoperitoneale e sottocutanea di *Candida albicans* e di altri lieviti asporigeni virulenti ed avirulenti in animali sottoposti a trattamento cortisonico.

Interessanti quadri morbosi sostenuti da *Candida* e da altre specie di miceti lievitiformi, sono stati descritti da diversi ricercatori in questi ultimi anni.

PORTELLA (52) e BUSSOLATI (7) segnalano casi di meningite e di meningoencefalite rispettivamente da *Criptococcus neoformans* e da *Mycotorula*. MALANDRA (44) descrive una candidiasi delle vie aeree superiori e dell'apparato gastroenterico insorta nel corso di una agranulocitosi.

Le candidiasi polmonari sono state oggetto di studio da parte di SAGGESE & GELLI (54), BRUSORI (6), GELLI (32) e FLORENZANO & ZINI (26) e sono ampiamente trattate in due interessanti monografie di ZINI (61) e di CORTI & CIARPAGLINI (20).

DE MARIA & TERAMO (22) descrivono un caso di micosi da *Candida* del colon sinistro.

La frequenza del reperto di *Candida* nell'escreato di tubercolotici e di altri pneumopatici ed il suo rapporto con l'andamento della malattia è stata studiata da BESTA e coll. (5) e da PALLINI & SBRANA (47) ed è stata discussa da FAZIO e coll. (24) i quali mettono in dubbio la patogenicità dei miceti isolati.

Il problema delle micosi insorgenti nel corso di trattamenti antibiotici è stato approfondito sul piano clinico da Ingrao (36), Sorice (55), Cottini (21), Arditi & Nigro (3), Forni (27), Masdea (45) e su quello sperimentale da Ambrosioni & Masoni (1, 2), Gargani & Fissi marracini (31), Vanini & Lostia (57).

Pisacane (51) descrive un caso di micosi del cavo orofaringeo a tipo pseudotubercolare sostenuto da *Monilia tropicalis*, Rizzi (53) una dermatosi blastomicetica nodulo gommosa diffusa e Vivarelli (59) una micocheratosi palmoplantare da *Mycothorula albicans*. Un caso di uretrite da *Candida* è stato descritto da Granelli (35).

Leone (39, 40, 41) ha accuratamente studiato la lipofilia e la patogenicità di *Pityrosporum ovalis* discutendo il significato che la sua presenza può avere nella genesi della pitiriasi del cuoio capelluto e dell'exema seborroico figurato. Il micete, che viene trovato nel 100 % dei soggetti sani e non è patogeno, non interviene direttamente nella genesi delle dermatosi seborroiche.

Bibliografia

1. Ambrosioni, P. & Masoni, S.: Ricerche sperimentali su conigli sani ed infettati con Candida albicans sottoposti a trattamento con clorotetraciclina (aureomicina). Nota II°. Tentativi di profilassi mediante somministrazione di vari gruppi di vitamine e germi del gruppo enterico. Boll. Ist. Sier. Milan. **34,** 418, 1955.
2. Ambrosioni, P., Masoni, S. & Zuliani, A.: Ricerche sperimentali in vivo sull'interferenza esplicata in vivo dalla Candida albicans sul potere antibatterico di alcuni antibiotici. Nuovi Ann. Igien. Microb. **6,** 265, 1955.
3. Arditi, E. & Nigro, N.: Considerazioni su di un caso di micosi da Candida albicans in soggetto immaturo. Giorn. Mal. Infett. Parass. **8,** 391, 1956.
4. Barbazza, M.: Azione dell'idrocortisone nelle micosi sperimentali da Candida. Med. Sper. **26,** 107, 1955.
5. Besta, B., Valenti, S., Cardarelli, E. & Leggeri, A.: Isolamento di forme micotiche del secreto bronchiale di pazienti affetti da malattie varie dell' apparato respiratorio. Ann. Ist. Forlanini **2,** 101, 1954.
6. Brusori, G.: Su di un caso di saccaromicosi polmonare. Ann. Rad. Diagn. **25,** 113, 1953.
7. Bussolati, C.: Un caso di meningoencefalite da Mycothorula. Giorn. Mal. Inf. Parass. **1,** 163, 1949.
8. Caputi, F.: L'azione penicillinasica e streptomicinasica della Mycothorula albicans. Boll. Soc. Ital. Biol. Sper. **28,** 1774, 1952.
9. Caputi, F.: Mycothorula albicans come antagonista della penicillina e della streptomicina. Boll. Ist. Sier. Milan. **31,** 315, 1952.
10. Caputi, F.: Dissociazione microbica da lisozima (Saggi sulla Mycothorula albicans). Boll. Soc. Ital. Biol. Sper. **28,** 141, 1952.
11. Caputi, F.: Antagonismo in vitro tra alcuni schizomiceti e mycothorula albicans. Boll. Soc. Ital. Biol. Sper. **32,** 532, 1956.
12. Castello, L.: Conoscenze attuali sull'azione batterica degli ultrasuoni e ricerche sperimentali sulla distruzione dei lieviti. Osservazioni preliminari. Giorn. Batt. Imm. **43,** 124, 1951.
13. Cazzola, D.: Ricerche sulla flora micotica dell'apparato genitale femminile. Nota IV°. Prove sperimentali sul comportamento quantitativo degli ifomiceti durante lo stato puerperale. Mon. Ost. Ginec. **19,** 35, 1948
14. Cazzola, D. & Mosconi, G.: Gli ifomiceti ed i saccaromiceti nella vagina della neonata e della donna impubere. Arch. Ital. Ost. Ginec. **52,** 65, 1947.

15. Cazzola, D. & Orlandini, E.: Contributo sperimentale alla conoscenza delle variazioni quantitative dei saccaromiceti nella vagina umana durante lo stato puerperale. Riv. Ost. Ginec. **69,** 425, 1947.
16. Cazzola, D. & Orlandini, E.: Ricerche sulla flora micotica dell'apparato genitale femminile. Nota III°. Ricerche qualitative sul secreto vaginale di donna con breve saggio di sistematica fungina. Ann. Ost. Ginec. **69,** 106, 1947.
17. Cazzola, D. & Orlandini, E.: Considerazioni sulla flora saccaromicetica ed ifomicetica nella vagina della donna. Folia Gynec. Pavia. **42,** 491, 1947.
18. Cipriani, P.: Comportamento dei blastomiceti di fronte al metodo di colorazione proposto da Dold. Mycopathologia. **4,** 187, 1948.
19. Colonnello, F.: Il significato biologico patologico di alcuni miceti lievitiformi nell' ambiente intestinale dell'uomo. Riv. Clin. Ped. **41,** 472, 1943.
20. Corti, L. & Ciarpaglini, L.: Le stafilocandidiasi broncopolmonari. Sett. Med. Edit. Firenze. 1955.
21. Cottini, G. B.: Antibiotici e micosi. Gazz. Med. Sicil. **2,** 1957.
22. de Maria, A. & Teramo, M.: Micetoma del colon sinistro. Policlinico. S.P. **61,** 1419, 1954.
23. di Bella, A.: Costanza del fenomeno di interferenza sull'azione antibatterica dei nuovi antibiotici in presenza di miceti. Nuovi Ann. Igien. Microb. **7,** 65, 1956.
24. Fazio, M., Minetto, E. & Oddone, I.: La broncomoniliasi esiste? Ann. Otorin. **71,** 666, 1954.
25. Ferraris, F.: Azione antibiotica di Oospora virescens sui funghi in medicina. Giorn. Batt. Imm. **45,** 108, 1953.
26. Florenzano, G. & Zini, F.: Osservazioni sperimentali sull'etiologia di una blastomicosi polmonare granulomatosa. Acc. Med. Fiorentina. 1950.
27. Forni, P. V.: Superinfezioni micetiche da antibiotici. Attività della colimicina e di altri antibiotici nelle candidiasi. Minerva. Med. Suppl. **77,** 828, 1956.
28. Forni, P. V.: Micosi sperimentali da Candida. Nota I°. Patogenicità e ripartizione della C. albicans nell'infezione sperimentale del topo albino. Med. Sper. **23,** 593, 1952.
29. Forni, P. V., Cauda di Puma, A. & Maghenzani, P.: Aminoacidossidasi dei miceti del genere candida. Med. Sper. **26,** 11, 1955.
30. Gargani, G.: Osservazioni sulla virulenza della Candida albicans. Giorn. Mal. Infett. Parass. **9,** 317, 1957.
31. Gargani, G. & Fissi Marraccini, G.: Isolamento e caratteri di patogenicità di C. albicans isolata in affezioni polmonari trattate con antibiotici e ricerche sierologiche sulla incidenza delle infezioni da C. albicans sulla popolazione normale. Lo Sperimentale. **106,** 102, 1956.
32. Gelli, G.: Micosi (Candidosi) polmonare in forma cavitaria (particolare riferimento al trattamento chirurgico). Riv. Clin. Ped. **54,** 155, 1954.
33. Gigli, L.: Studio della resistenza crociata su ceppi di candida albicans con resistenza indotta verso alcune sostanze coloranti. Arch. Ital. Derm. **28,** 133, 1956.
34. Gigli, L.: Azione in vitro di alcune sostanze coloranti verso la Candida. Arch. Ital. Derm. **27,** 209, 1955.
35. Granelli, U.: Sopra un caso di uretrite di origine micosica. Minerva. Derm. **30,** 191, 1955.
36. Ingrao, F.: Micosi e infezioni batteriche secondarie e terapie antibiotiche. Rec. Progr. Med. **13,** 325, 1952.
37. Lanzara, A.: Micosi sperimentali del fegato da Mycothorula albicans. Arch. Med. Sper. **17,** 356, 1946.
38. Lanzara, A.: Ricerche sperimentali sulla micosi della lingua da Mycothorula albicans. Arch. Med. Sper. **17,** 345, 1946.
39. Leone, R.: Presenza e significato del Pityrosporum ovalis nella pitiriasi del cuoio capelluto nell'exema seborroico figurato ed in dermatosi squamose varie. Nota III°. Minerva Derm. **27,** 123, 1952.
40. Id. Id. Nota I°. Minerva Derm. **27,** 93, 1952.
41. Id. Id. Nota IV°. Minerva Derm. **27,** 128, 1952.

42. Magni, G. E.: Analisi della ploidia di alcuni generi di lieviti asporigeni. Atti Ist. Bot. Pavia, **10,** 89, 1953.
43. Magni, G.: Biological significance of the pseudomycelium in asporogenous yeast. I⁰. Reciprocal inhibition of parallel colonies. Mycopathologia. **4,** 210, 1947.
44. Malandra, B.: Di un caso di malattia ,,Mughetto'' nell'adulto. Riv. Anat. Pat. Oncol. **1,** 385, 1949.
45. Masdea, E.: Su un caso di moniliasi polmonare. Arch. Ital. Sc. Med. Trop. **35,** 243, 1954.
46. Masoni, S. & Papinutto, G. P.: Ulteriore contributo alla interferenza esplicata in vitro dalla candida albicans sul potere antibatterico di alcuni antibiotici. Nuovi Ann. Igien. Microb. **6,** 265, 1955.
47. Pallini, V. & Sbrana, E.: Osservazioni cliniche e di laboratorio sulle micosi polmonari primitive e associate a Tbc polmonare. Boll. Soc. Med. Chir. **22,** 400, 1954.
48. Piantoni, L.: Azione del cortisone sulle infezioni sperimentali da lieviti asporigeni avirulenti e virulenti. Giorn. Mal. Infett. Parass. **7,** 247, 1955.
49. Piantoni, L.: Azione allobioergica del cortisone sull'infezione sperimentale da Debaromyces neoformans. Biologia Latina. **8,** 997, 1955.
50. Piantoni, L. & Sirtori, G.: Contributo allo studio della patologia sperimentale da Debaromyces neoformans. (Sanfelice) red. Cif. Giorn. Biologia Latina. **8,** 953, 1955.
51. Pisacane, C.: Su di un caso di micosi del cavo orofaringeo e dell'apparato respiratorio a tipo pseudotubercolare. Ann. Ital. Derm. **4,** 105, 1949.
52. Portella, A.: Su di un caso di meningite da Torulopsis neoformans. Studio del micete isolato. Riv. Ist. Sier. Ital. **28,** 524, 1953.
53. Rizzi, V.: Blastomycosis acneoides conglobata. Arch. Ital. Derm. **23,** 321, 1950.
54. Saggese, V. & Gelli, G.: Contributo alla conoscenza delle micosi polmonari del bambino. Riv. Clin. Ped. **51,** 182, 1953.
55. Sorice, F.: Superinfezioni micotiche in corso di terapia antibiotica. Clin. e Ter. **6,** 12, 1954.
56. Urso, B.: Nuovo contributo alla broncomoniliasi sperimentale. Arch. Ital. Sc. Med. Col. **31,** 77, 1950.
57. Vanini, G. & Lostia, G. B.: Ricerche sperimentali sull'azione degli antibiotici su C. albicans. Igiene Mod. **49,** 1125, 1956.
58. Verona, O. & Florenzano, G.: Sopra alcuni aspetti morfologici di Candida pulcherrima (Lindner) Windisch. Mycopathologia. **4,** 268, 1948.
59. Vivarelli, I.: Micocheratosi palmare e plantare con onicomicosi da Mycothorula albicans. Giorn. Ital. Derm. **90,** 1, 1949.
60. Zambotti, V. & Moret, V.: Studi sulla biochimica dell'Oospora lactis. III⁰. Capacitá di sintesi dei protidi a spese di azoto organico. Boll. Soc. Ital. Biol. Sper. **25,** 779, 1949.
61. Zini, F.: Le candidasi del polmone. Lumini Ed. Firenze. 1953.
62. Zini, F.: Sulla diffusione dei lieviti patogeni nella membrana corionallantoidea e testacea dell'embrione di pollo. Giorn. Batt. Imm. **44,** 329, 1952.

III. ASPERGILLI, PENICILLI E MICOSI DA ESSI DETERMINATE

Villa (8) ha potuto dimostrare che *Aspergillus fumigatus* elabora sostanze ad azione antidotica e Tedeschi (7) che i pigmenti da *Aspergillus niger* esplicano un'attività antiprotozoaria. Dalle ricerche di Rossi & Jacoli (4) risulta che *Aspergillus niger* è capace di effettuare, in mezzo culturale idoneo, la sintesi totale della tiamina. Russo & Graziosi (5) inoculando per via endovenosa *Aspergillus nidulans*, ottengono negli animali da esperimento lesioni nodulari necrotiche del fegato e del polmone.

Dal punto di vita nosografico sono da segnalare alcuni casi di otomicosi da *Aspergillus versicolor* descritti da DE AMICIS (1), uno di micosi ossea da *Aspergillus herbariorum* osservato da SPEDIACCI (6) e due casi di micosi polmonare da *Aspergillus* di specie non identificata documentati da REDAELLI e coll. (3).

MAGNI & VILLA (2) hanno dimostrato che *Penicillium cloroleucum* produce un principio antibiotico attivo su *V. cholerae, S. typhi* e *Dis. Shiga.*

Bibliografia

1. DE AMICIS, E.: Osservazioni cliniche, micologiche e patogenetiche su tre casi di otomicosi da Aspergillus versicolor. Boll. Mal. Orecchio. **68**, 278, 1950.
2. MAGNI, A. & VILLA, A.: Antibiotic substances and control of gramnegative microrganism: Activity of Penicilium chloroleucum. Mycopathologia. **4**, 213, 1947.
3. REDAELLI, P., ROSSI, L. & PALAZZI, D.: Broncopneumomicosi aspergillari nell'unomo e trattamento antibiotico. Arch. Ital. Ist. Anat. Pat. **28**, 329, 1954.
4. ROSSI, C. A. & JACOLI, G. C.: Ricerche sulla sintesi di tiamina da Aspergillus niger. Boll. Soc. Ital. Biol. Sper. **25**, 826, 1950.
5. RUSSO, G. & GRAZIOSI, F.: Sul potere patogeno sperimentale dell'aspergillus nidulans. Rend. Ist. Sup. San. Roma. **13**, 46, 1950.
6. SPEDIACCI, G.: Sopra un caso di sospetta micosi del piede da aspergillo. Minerva Ort. **2**, 231, 1951.
7. TEDESCHI, G. C.: Sull'attività antiprotozoaria dei pigmenti dell'Aspergillus niger. Boll. Soc. Ital. Biol. Sper. **24**, 923, 1948.
8. VILLA, A. M.: Ricerche su sostanze ad azione antidotica prodotte da miceti (Attività dell'Aspergillus fumigatus). Mycopathol. et Mycol. appl. **5**, 1. **1951**.

IV. ACTINOMICETI ED ACTINOMICOSI

Proseguendo nelle ricerche che da anni conduce sull'argomento BALDACCI (2, 3, 4, 5, 6, 7, 9, 10) ha elaborato uno schema classificativo degli actinomiceti che permette di classificare le diverse specie in base al colore del micelio vegetativo e del micelio aereo. Tale schema ha consentito a BALDACCI di ordinare gli actinomiceti in serie che, pur non costituendo un gruppo sistematico qualificato, ma solo un arbitrario raggruppamento, facilitano il lavoro di classificazione in corso di sviluppo. Della classificazione degli actinomiceti si sono occupati autorevolmente anche CIFERRI (12) e PUNTONI (25).

Il ciclo vitale degli actinomiceti è stato studiato per mezzo del microscopio elettronico da BALDACCI e coll. (8).

GIOLITTI & BERTANI (17) hanno elaborato, per lo studio microcolturale degli actinomiceti, una interessante metodica basata sull'impiego di un supporto di cellophane; POLLACCI (23) si è occupato dei substrati atti alla preparazione di streptomicina da *Actinomyces griseus* e BORETTI e coll. (11) delle variazioni morfologiche e biologiche di *Streptomyces aureofaciens* in rapporto alla produzione di clorotetraciclina. FLORESTANO (14) ha studiato l'azione fitostatica di *Actinomyces albus* e *A. griseus.*

La virulenza degli actinomiceti è stata studiata da PIANTONI (22) in rapporto con la somministrazione di cortisone. SQUADRINI e coll. hanno potuto ottenere una tireopatia sperimentale nel ratto inoculando direttamente nell'organo *Actinomyces bovis.*

Casi di actinomicosi polmonare sono stati descritti da GERIOLA (15) e da SCARINCI (28), di meningite actinomicotica da TUCI (31) e da MENGHI e coll. (21). Interessanti osservazioni di actinomicosi cutanea e viscerale da Actinomyces di specie diverse (*A. hodeidae, A. israeli,* ecc.) sono state riportate da diversi ricercatori (1).

Bibliografia

1. AMBROSIONI, P. & MERUCCI, E. L.: Micetoma a grani rossi ad Hodeida nello Yeman. Riv. Parass. **11,** 213, 1942.
2. BALDACCI, E.: Die Systematik der Actinomyceten. Mycopathologia, **4,** 60, 1947.
3. BALDACCI, E.: Sulla corretta diagnosi del genere actinomyces e della specie bovis. Ann. Igiene e Microb. **2,** 230, 1951.
4. BALDACCI, E.: Revisione delle specie: Actinomyces albus; A. cromogenes; A. odorifer; A. termophylus; A. viridis; A. viridocromogenes; A. hominis; A. innominatus. Mycopathologia. **2,** 145, 1940.
5. BALDACCI, E.: Contributo alla sistematica degli actinomiceti. IX⁰. Saggio di una classificazione e critica al concetto di spora degli Actinomycetales. Atti Ist. Bot. Pavia. **13,** 99, 1941.
6. BALDACCI, E. & COMASCHI, F.: Contributo alla sistematica degli actinomiceti. XVIII⁰. Actinomyces griseus (Krainsky) Waksmann. Mycopathol. et Mycol. appl. **7,** 278, 1954.
7. BALDACCI, E., COMASCHI, G. F., SCOTTI, T. & SPALLA, C.: General criteria for the systematics of genera and species of Actinomyces (Streptomyces) and Micronospora. Ist. Sup. San. **11,** 20, 1953.
8. BALDACCI, E., GILARDI, E. & AMICI, A. M.: Il ciclo di vita degli Actinomiceti osservato al microscopio elettronico. Giorn. Microb. **1,** 512, 1956.
9. BALDACCI, E. & SPALLA, C.: Contributo alla sistematica degli actinomiceti. XVII⁰. Actinomyces scabies (Taxer) Waksmann. Mycopathol. et Mycol. appl. **7,** 269, 1954.
10. BALDACCI, E., SPALLA, C. & GREIN, A.: La classificazione delle specie di actinomyces (Streptomyces). Ann. Microb. **6,** 41, 1954.
11. BORETTI, C., DI MARCO, SCOTTI, & ZOCCHI.: Variazioni morfologiche e biologiche dello Streptomyces aureofacies in relazione alla produzione di clorotetraciclina. Giorn. Microb. **1,** 97, 1955.
12. CIFERRI, R.: Biology and taxonomy of the actinomycetes. Atti Ist. Bot. Pavia. **10,** 187, 1953.
13. CILLI, V., BATTELLI, C. & CECCARELLI, A.: Ricerche sperimentali su di uno stipite di Asteroides (Proactinomyces, Nocardia) asteroides repertato in un caso di actinomicosi spontanea del cane. Boll. Ist. Sier. Milan. **23,** 139, 1954.
14. FLORENZANO, G.: Comportamento fitostatico di Actinomyces albus e A. griseus. Ric. Sc. Ricostr. **17,** 2039, 1947.
15. GERIOLA, F.: Su di un caso di actinomicosi polmonare trattato con streptomicina. Giorn. Mal. Infett. Parass. **1,** 247, 1949.
16. GIAMPICCOLO, P.: Actinomicosi cervico-facciale da disodontiasi di un terzo molare inferiore. Riv. Ital. Stom. **7,** 1020, 1950.
17. GIOLITTI, G. & BERTANI, M.: A method for the microscopical study of actinomycetes. J. Bact. **65,** 281, 1953.
18. LEONE, R.: Contributo alla conoscenza dell'actinomicosi cutanea da aerobi. Minerva Derm. **12,** 355, 1956.
19. MACONI, G.: Su di un caso di actinomicosi del collo trattato con aureomicina. Rass. Int. Clin. Terap. **33,** 279, 1953.
20. MARAGNANI, U.: Su di un caso atipico di actinomicosi. Giorn. Ital. Derm. **89,** 1232, 1948.

21. Menghi, P. & Minazza, R.: Su di un caso di meningite da Actinomiceti. Giorn. Mal. Inf. Parass. **8**, 526, 1956.
22. Piantoni, L.: Azione del cortisone sulle infezioni sperimentali da actinomiceti avirulenti e virulenti. Biol. Latina. **8**, 1095, 1955.
23. Pollacci, G.: Sul terreno atto a coltivare l'actinomyces griseus ed a preparare la streptomicina. Boll. Soc. Med. Chir. Pavia. **63**, 379, 1949.
24. Puccinelli, V. A.: Proactinomicosi del volto. Giorn. Ital. Derm. **89**, 92, 1948.
25. Puntoni, V.: Gli actinomiceti nella patologia umana e veterinaria. Zooprofilassi. **8**, 20, 1953.
26. Redaelli, P. & Piantoni, L.: La virulenza degli actinomiceti. Giorn. Mal. Infett. Parass. **5**, 77, 1953.
27. Scalfi, A.: Sul flemmone ligneo actinomicotico perirenale clinicamente primitivo. Ann. Ital. Chir. **26**, 15, 1950.
28. Scarinci, C.: Actinomicosi polmonare primitiva; identificazione del parassita mediante broncoaspirazione, guarigione con l'associazione penicillina sulfamidici a forti dosi. Policlinico. **60**, 814, 1953.
29. Severi, R. & Amistani, B.: Su di un singolare reperto di actinomicete anaerobio presente in un caso di actinomicosi umana unitamente al bacterium actinomycetemconcomitans e simulante nelle colture Streptobacillus moniliformis. Atti Soc. Med. Chir. Padova. **24**, 111, 1946.
30. Squadrini, F. & Berengo, A.: Tireopatia sperimentale da inoculazione intratiroidea di Actinomyces bovis nel ratto normale. Riv. Ist. Sier. Ital. **27**, 485, 1952.
31. Tuci, P.: Il quadro istopatologico delle leptomeningiti actinomicosiche nella diagnostica differenziale delle leptomeningiti di aspetto purulento. Arch. De Vecchi. **5**, 933, 1943.

V. COCCIDIOIDOMICOSI

Cirenei (1) studiando l'andamento dell'infezione sperimentale da *Coccidioides immitis* ha messo in evidenza che la malattia decorre negli animali tiroidectomizzati in modo più grave che in quelli normali.

Redaelli e coll. (2) hanno potuto documentare che nei ratti trattati con cortisone l'infezione sperimentale da *Coccidioides immitis* evolve in modo nettamente diverso dai controlli. Alla inoculazione sottocutanea segue una diffusione viscerale della infezione e nella sede d'impianto la reazione tissutale anzichè di tipo granulomatoso appare di tipo necrotico essudativo.

Bibliografia

1. Cirenei, A.: Contributo allo studio di una micosi granulomatosa (coccidioidomicosi). La micosi negli stati di modificata funzionalità tiroidea. Boll. Soc. Ital. Biol. Sper. **23**, 20, 1947.
2. Redaelli, P., Cavallero, C., Borasi, M., Sala, G. & Amira, A.: Infezione sperimentale da coccidioides immitis e steroidi corticosurrenali. Mycopathol. et Mycol. appl. **6**, 7, 1953.

VI. BLASTOMICOSI SUDAMERICANA

Farris (1) ha descritto un caso di blastomicosi sudamericana particolarmente interessante per la rarità del reperto in Italia e per alcuni suoi caratteri clinici quali il decorso prolungato, la presenza di lesioni lichenoidi, la marcata componente eosinofila delle lesioni granulomatose e la resistenza alla terapia.

Bibliografia

1. FARRIS, G.: Contributo personale alla conoscenza della paracoccidioidomicosi (cosidetta blastomicosi brasiliana). Giorn. Ital. Derm. **96,** 321, 1955.

VII. SPOROTRICOSI

Un caso di lesioni gommose non ulcerate del piede da cui viene isolato *Sporotrichum beuermanni* è stato descritto da CAMPANELLA (1). SANTORI (3) ha segnalato un caso di sporotricosi a tipo linfangitico e RESTA (2) una dermatomicosi ulcero-gommosa ed ulcero-vegetante da cui viene isolato *Sporotrichum epigaeum.*

Bibliografia

1. CAMPANELLA, P.: Su di un caso di sporotricosi. Riforma Med. **63,** 692, 1949.
2. RESTA, V.: Sporotricosi verrucosa e gommosa a quadro clinico blastomicosico. Minerva Derm. **26,** 66, 1951.
3. SANTORI, G.: Osservazioni su due casi di micosi cutanee. Ann. Ital. Derm. **4,** 272, 1949.

VIII. ISTOPLASMOSI

L'osservazione di alcuni casi clinici (8) particolarmente interessanti ha permesso di documentare l'esistenza anche in Italia della Istoplasmosi ed ha indotto alcuni ricercatori a studiare diversi problemi inerenti a questa malattia. CARAVAGLIOS (1), FOIS (4), BUONOCORE (3), TASSINARI (10), PARRELLA (6) e RALLO (7) si sono occupati della nosografia e della epidemiologia dell'istoplasmosi e BRACCINI (2), TASSINARI (9) e FOIS (4) della reattività cutanea all'istoplasmina.

Bibliografia

1. CARAVAGLIOS, F.: L'importanza dell'istoplasmosi. Rass. Int. Clin. Ter. **32,** 556, 1952.
2. BRACCINI, L.: Sulla incidenza dell'istoplasmosi in alcune provincie della Toscana. Riv. Ital. Igiene. **12,** 429, 1952.
3. BUONOCORE, P.: L'istoplasmosi. Pediatria. **58,** 792, 1950.
4. FOIS, A.: Richerche preliminari sulla cutireattività all'istoplasmina e alla coccidioidina in un gruppo di bambini della Toscana. Riv. Clin. Ped. **51,** 840, 1953.
5. FOIS, A.: La istoplasmosi, coccidioidomicosi, blastomicosi e moniliasi nella pratica pediatrica. Riv. Clin. Ped. **52,** 452, 1953.
6. PARRELLA, M.: L'Istoplasmosi polmonare. Arch. Tisiol. **7,** 237, 1952.
7. RALLO, A.: Nosografia ed epidemiologia dell'istoplasmosi. Rif. Med. **66,** 775, 1952.
8. SOTGIU, G. & CORBELLI, G.: Micosi rare. Osservazioni dei due primi casi di istoplasmosi in Italia e di un caso di coccidioidomicosi. Boll. Sc. Med. **127,** 85, 1955.
9. TASSINARI, G.: La sensibilità cutanea all'istoplasmina: primi risultati in Italia. Nota preventiva. Sett. Med. **36,** 90, 1948.
10. TASSINARI, G.: In tema di micosi: aggiornamento sull'istoplasmosi di Darling. Sett. Med. **39,** 225, 1951.

LIBRI NOVI

KASHKIN, P. N. (1958): Candidosy (vozbuditeli, klinika, i epidemiologia). [Candidoses (causative agents, clinical data, and epidemiology)]. Medgiz. Leningrad, 271 pp. 11 R. 40 kop.

The author, "meritorious man of science" and Stalin prize laureate, presents a monograph of the Candidoses, i.e. those diseases that are provoked by yeastlike fungi of the genus *Candida*, also often called Moniliases in the literature. After a short introduction concerned mainly with the history of the subject, there is a chapter on the morphology and biology of the genus *Candida* which treats the general characteristics of the morphological characters, the cultural characters, physiology, variability, constancy and resistance against physico-chemical and biological factors, pathogenity of the yeast-like species of *Candida*, antigens, antibodies and the allergic forms of the candidoses, immuno-biological reactions in candidoses, finally principles of the classification of the yeast-like fungi of the genus *Candida*.

The second chapter treats the pathogenesis and the clinical aspects of candidoses, reproduces the classification of candida-infections by A. N. ARAVYSKY and treats the various and very numerous forms subsequently, viz. infection of the skin, the mucous membrane, of the internal organs, the allergic forms of candidoses and adds data on professional candidoses and on candidosis complications of the antibiotics therapy. The last subject of the chapter refers to the therapy of the Candidoses.

There follows a chapter on laboratory diagnosis (microscopical investigation, cultural diagnostics, study on the biochemical activity of these fungi, determination of the pathogenicity, and the serological diagnostic of candidoses as well as allergy tests, histopathological investigations in presence of candidoses and the characteristics of the most common yeast-like fungi of the genus *Candida* (differentiating them according to the six groups: *albicans, tropicalis, pseudotropicalis, guillermondii, krusei, brumptei*).

The last chapter on epidemiological data is followed by a rather extensive list of Russian and foreign literature.

The text is completely in Russian. It is illustrated by 32 text figs. not all of them very clearly reproduced but mostly sufficiently illustrative with regard to the related passages of the text.

The author has used the foreign literature at his disposal, the very rich Soviet literature on the clinical and experimental phase of the problem, and expresses, beyond this, the practical experience of his pupils and collaborators as well as in some instances his own critical view of certain problems. The monograph approaches the whole problem from very different angles (without losing itself in detail) — although KASHKIN modestly says that he did not pretend to present an exhaustive treatment of all questions related to the problem of Candidoses. The book is written for physicians.

ROLF SINGER

Department of Botany, Instituto M. Lille,
Universidad Nacional de Tucumán
Argentina

SEELIGER, H. P. R.: *Mykologische Serodiagnostik* (Mycological sero-diagnosis). Beiträge zur Hygiene und Epidemiologie, Heft 11, 194 p. J. A. Barth Verlag, Leipzig, 1958. Price: DM 20.-

The need for such a monographic presentation as this publication on the serodiagnosis of fungus diseases was felt for a long time and was, indeed, overdue. After discussing the basic methodology of fungus serology, the author gives the detailed technic and methods of the serologic tests, precipitation, agglutination, complement fixation, etc. Separate chapters deal with the serology of fungi (hyphomycetes, yeast-like organisms) and the serology of the mycoses themselves. The well balanced presentation excels in its objectivity, clearly pointing out what we can expect from fungus serology as diagnostic aid and what we can not. This is a great merit of this monograph. It is well written, in a clear, light understandable language. The black and white illustrations (82 figures) are well chosen and underscore the point the author wanted to transmit. The bibliography (516 items!) is most comprehensive and wholly international. It is a great service to all those — mycologists, clinicians and laboratory workers — for whom this work is simply indispensable. The make-up of the monograph (paper, print, etc.) is excellent. It can be warmly recommended.

TIBOR BENEDEK

CIFERRI, R.: G. Lindau and P. Sydow's Thesaurus Literaturae Mycologicae et Lichenologicae Supplementum, 1911—1930. Vol. 3, L—Q. pp. 1477—2156. Renzo Cortina, Pavia, Italy.

The third volume of this important 4-volume bibliography was printed in December 1958 and issued in February 1959. It includes authors from L through Q. Reference numbers 15296—22370 are included.

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

WM. BRIDGE COOKE

VAN DER WERFF, P. J.: Mould Fungi and Bronchial Asthma. I. Linen, 174 pages, 67 figures in black and white. Charles C Thomas, Publisher Springfield, Illinois, 1958. Price: $7.50.

This first volume of the monograph closes a long felt gap in the mycological literature. Written by an internist (allergologist) with well founded mycological knowledge, he critically reviewed the extremely dispersed material on the subject from the world literature. It goes without saying that the basis of the whole monograph is founded in his own clinical and laboratory investigations carried out with remarkable assiduity.

This monograph contains five chapters. It reviews investigations into climate and fungus allergy, underscoring the particular merits of the outstanding Dutch pioneer investigator in the field, STORM VAN LEEUWEN. Next, a survey of general mycology follows with classification of fungi and with emphasis on special methods in the investigation of mould spores in the open air. He stresses the fact that it is essential to use natural nutrient media in cultivating, isolating and identifying the fungi. The third chapter deals with mould spores in the open air, while the fourth one covers the fungi found in houses. The last chapter investigates the fungi in factories and workshops.

The investigation is based on studies of frequency distribution of fungus spores in the outdoor air in the Central and Western parts of the Netherlands. These data are carefully compared with findings in the Northern part of the country (Groningen) and also with the principal data relating to other countries. The technical and mycological data have been given in an attempt to guide others in persuing their studies in this field.

Beside numerous graphs showing the frequency distribution of airborn fungi (spores) in different cities, there are 67 black and white photographs and photomicrographs depicting the microscopic features of the fungi mainly responsible as allergogens. The book is produced in the Netherlands with the well known mastery of Dutch book printing. In the page mirror the use of the different types helps a great deal for quick orientation. A comprehensive bibliography is added. There is a list of the authors. Subject index is missing which would have enhanced the usefulness of this work.

The author solved a difficult task with great skill and erudition. This monograph is a must in the laboratory and library of allergologists, mycologists and even meteorologists.

A forthcoming second volume shall discuss the problem as to whether or not there is a generic and/or specific fungus allergy.

TIBOR BENEDEK

ICONOGRAPHIA MYCOLOGICA

Onorato Verona & Tibor Benedek

Istituto di Patologia Vegetale e di Microbiologia Agraria e Tecnica dell' Università di Pisa

Mycology Section, Department of Dermatology, Stritch School of Medicine, Loyola University, Chicago, Ill.

Mycopathologia et Mycologia Applicata is beginning a new feature from Volume XI on in form of supplements: **Iconographia Mycologica.** It intends to present fungi in four groups under the following headings: 1) Saprophyta; 2) Phytopathogens; 3) Zoopathogens and 4) those in Industrial Use. The first group, thus, will cover fungi vegetating in nature without any known pathogenicity. The fourth group will contain fungi, helpers in man's industrial activities. The second and third groups are self-explanatory. Every Plate will be accompanied by a short, concise description in order to make the graphical presentation that much more desirable and conducive to immediate understanding. Every supplement will bring Plates from any, or all of the four groups. They will be arranged so that they can separately be collected in folders under these four, separate headings or, eventually, under the great taxonomical groups, howsoever the reader considers them most suitable. We hope that this new feature will greatly enrich the broad coverage of subjects in **Mycopathologia et Mycologia Applicata.** Because of the great and remarkable interest at present that fungi have in human as well as in natural economy, **Iconographia Mycologica** shall familiarize the reader not alone with the wealth of beautiful, unsurpassed, artistic creation of nature but it shall try also to be of substantial help in the study and identification of these fungi.

The various drawings are not presented according to a fixed criterion or according to a fixed object. It would have been easy to limit ourselves to established taxonomical groups or to limit the presentation to phytopathogenic or zoopathogenic fungi alone. However, we preferred not to put any narrow limitation on our endeavor and to present occasionally also various other genera. The reason for this apparent disorder is the desire to present the greatest possible number of different morphological structures and to offer a challenge for better exercising one's judgment in observation of these structural forms as they pass from one taxonomical group to another.

Finally, we do not limit ourselves to present classical genera alone, but we want to bring — from time to time — also genera just recently described.

References

In the preparation of **Iconographia Mycologica** the following sources have been mainly consulted:

ALEXOPOULOS, C. J.: Introductory Mycology. J. Wiley and Sons, Inc. New York, 1952.

BARNETT, H. L.: Illustrated Genera of Imperfect Fungi. Burgess Publ., Minneapolis, 1956.

BESSEY, E. A.: A Textbook of Mycology. P. Blackiston's Son & Co. Philadelphia, Pa. Corrected reprint, 1939.

CLEMENTS, F. E. & SHEAR, C. L.: The Genera of Fungi. The H. W. Wilson Co., New York, 1957.

GILMAN, J.: A Manual of Soil Fungi. The Iowa State College Press, Ames, 1957.

GÄUMANN, E. A.: Comparative Morphology of Fungi. McGraw-Hill Book Co., New York, 1928.

GWYNNE-VAUGHAN, H. C. I.: Fungi (Morphology). 232 pp., 196 Figs., London, 1922.

GWYNNE-VAUGHAN, H. C. I. & BARNES, B.: The Structure and Development of the Fungi. University Press, Cambridge, 1937.

HUGHES S. J.: Conidiophores, conidia and classification - Can. J. Bot. 31, 577, 1953.

LINDAU, G.: Die Mikroskopischen Pilze. Bd. 2/1, 2 : 2 Auflage, Berlin, J. Springer, 1922.

SACCARDO, P. A.: Sylloge fungorum omnium hucusque cognitorum. Twenty-five volumes issued up to 1931. Pavia, Italy.

VIENNOT-BOURGIN, S.: Les Champignons Parasites des Plantes Cultivées. Masson & Cie, Paris 1949,

and some of the most important Reviews of Mycology enclosed **Mycological Papers** of the Commonwealth Mycological Institute.

ICONOGRAPHIA MYCOLOGICA

Verona—Benedek

LIST OF PLATES IN SUPPLEMENT I 1)

A. FUNGI IMPERFECTI

			Saprophyta	Phytopathogen	Zoopathogen	Industrial
1	Gen.	*Helicodendron* Peyr.	+			
		Helicomyces Link	+			
		Helicoon Morgan	+			
		Spirosphaera v. Beverwijk	+			
2	Gen.	*Monochaetia* Sacc.	+			
		Pestalozzia DeNot.	+	o		
3	Gen.	*Coryneum* Nees		+		
4	Gen.	*Helicosporum* Nees	+	o		
		Helicoma Cda.	+	o		
		Helicomina Olive	+	o		
5	Gen.	*Pachybasium* Sacc.	+			
		Verticillium Nees	+	o		
		Acrostalagmus Cda.	+			
6	Gen.	*Alternaria* Nees	+	o		
		Stemphylium Wallr.	+	o		
7	Gen.	*Sporotrichum* Link	+		o	
8	Gen.	*Trichophyton* Malm.	+		o	
9	Gen.	*Cephalothecium* Cda.	+	o		
		Didymopsis Sacc. & March.	+	o		
		Trichothecium Link	+	o		
		Mycogone Link	+	o		
10	Gen.	*Candida* Berkh.	+		o	o
11	Gen.	*Chalara* Cda.	+			
		Chalaropsis Peyron.	+			
12	Gen.	*Cercospora* Fres.		+		
13	Gen.	*Oidium* Link	+			
		Oidiopsis Scalia	+			
		Ovulariopsis Patouillard et Hariot	+			

---

1) The sign (+) means exclusively or prevalently; the sign (o) means limited only to some species.

B. PHYCOMYCETEAE

			Sapro-phyta	Phyto-pathogen	Zoo-pathogen	Indus-trial
1	Gen.	*Linderina* Raper et Fennel	+			
		Coemansia v. Thieg. et Le Monnier	+			
2	Gen.	*Blastocladiella* Matthews	+			
		Blastocladia Reinsch	+			
3	Gen.	*Mucor* Micheli	+			o
4	Gen.	*Brevilegnia* Coker & Couch	+			
5	Gen.	*Isoachlya* Kauffman	+	o		
		Achlya Nees von Esenbeck	+	o		
6	Gen.	*Thraustotheca* Humphrey	+			
		Dictyuchus Leitgeb.	+			
7	Gen.	*Syncephalastrum* Schroet.	+	o		
		Syncephalis van Thieg. & Le Mon.	+	o		

C. ASCOMYCETEAE

1	Gen.	*Sordaria* Ces. & DeNot.	+			
2	Gen.	*Chaetomium* Kze	+			o
3	Gen.	*Pleospora* Rabh.	+	o		
4	Gen.	*Coleroa* Rabh.	+	o		
5	Gen.	*Glomerella* Schrenk & Spauld.		+		
6	Gen.	*Sphaerotheca* Lev.		+		
		Podosphaera Kze.		+		
		Microsphaera Lev.		+		
7	Gen.	*Phyllactinia* Lev.		+		
		Erysiphe Hedw.		+		
8	Gen.	*Uncinula* Lev.		+		
9	Gen.	*Aspergillus* (Michel.) Link	+		o	o
10	Gen.	*Balladyna* Rac.	+	o		
		Balladynopsis Theiss & Syd.	+	o		
		Balladynella Theiss & Syd.	+	o		
		Dysrhynchis Clem.	+	o		
11	Gen.	*Lophiotrema* Sacc.	+			
		Vivianella Sacc.	+			
		Lophiostoma C. & DeNot.	+			
		Brigantiella Sacc.	+			
12	Gen.	*Eutypella* (Nke.) Sacc.	+	o		

D. PROMYCETES

1	Gen.	*Tilletia* Tul.		+		
2(a,b)	Gen.	*Puccinia* Pers.		+		
3	Gen.	*Phragmidium* Link		+		

FUNGI IMPERFECTI
MONILIALES
MONILIACEAE

SAPROPHYTA

Gen. Helicodendron PEYRONEL.

Gen. Helicomyces LINK

Gen. Helicoon MORGAN

Gen. Spirosphaera v. BEVERWIJK

ICONOGRAPHIA MYCOLOGICA
VERONA — BENEDEK
Plate A 1

Conidia spirally curved, hyaline or bright colored, cylindric

Conidia catenate, conidiophores and chains ramose

1 – Genus Helicodendron Peyr.

Conidia not catenate, concentrically coiled

2 – Genus Helicomyces Link

Conidia not catenate, spirally twisted into a conic or ovoid tube

3 – Genus Helicoon Morgan

Conidia curved sometime ramified

4 – Genus Spirosphaera v. Beverwijk

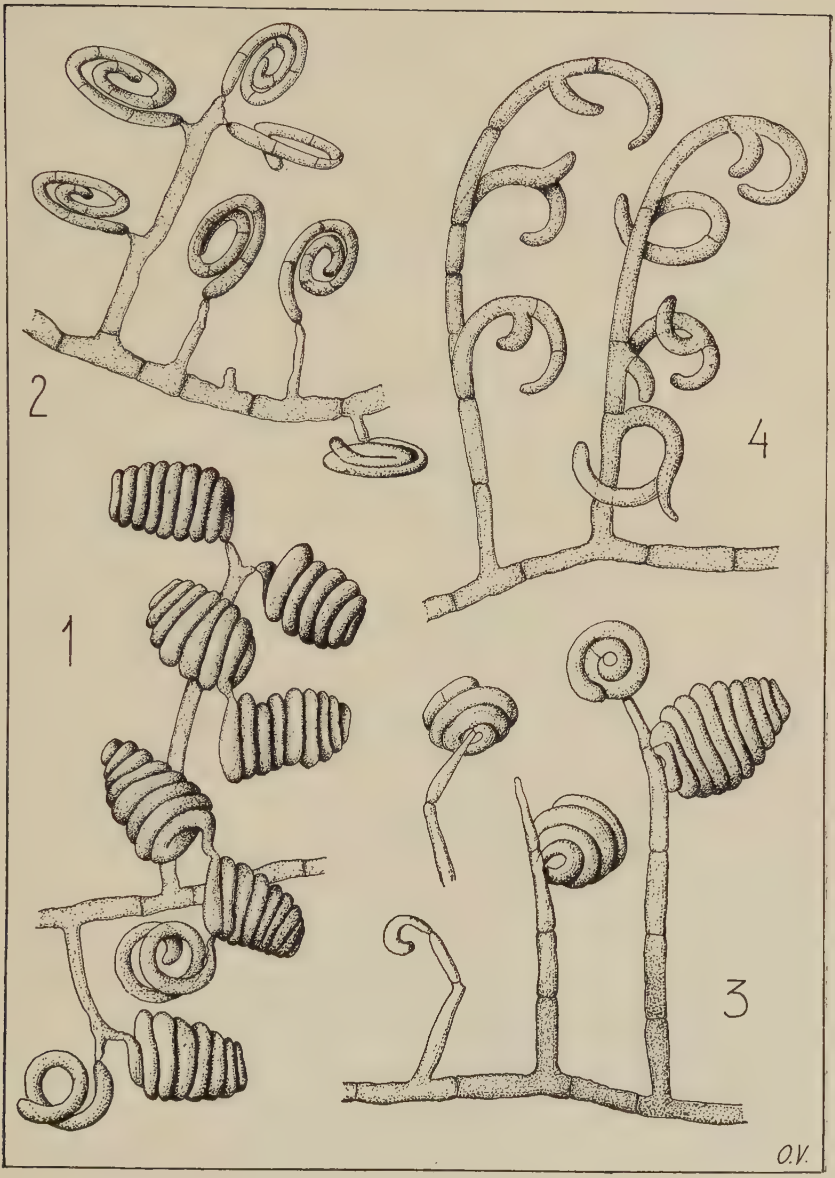


PLATE A 1

FUNGI IMPERFECTI
MELANCONIALES
MELANCONIACEAE

SAPROPHYTA
PHYTOPATHOGEN

Gen. Monochaetia SACC.

Gen. Pestalozzia DENOT.

ICONOGRAPHIA MYCOLOGICA
VERONA — BENEDEK
Plate A 2

Conidia not catenate, x-celled, ciliate at the apex:

1 – Genus Monochaetia

Conidia 1-ciliate above

2 – Genus Pestalozzia

Conidia 2-4-ciliate above

Acervulus and conidia.

Some species of this genus may be parasitic on plants.

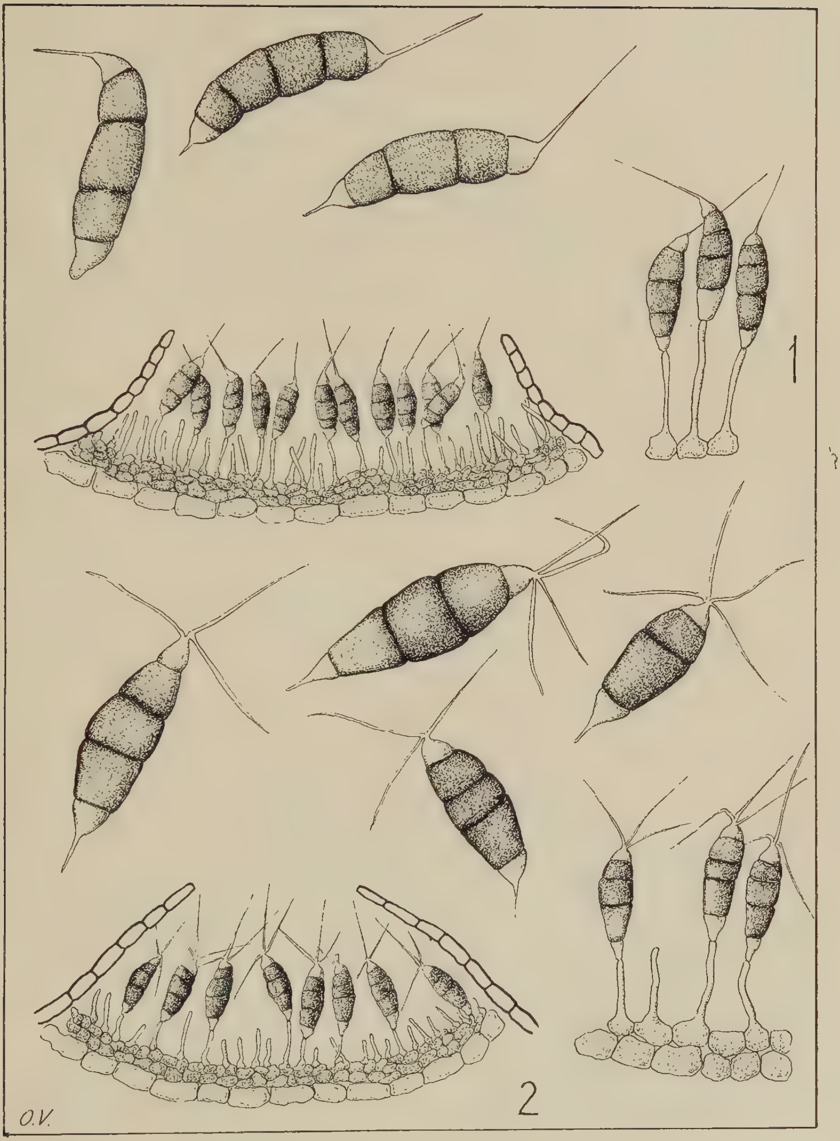


PLATE A 2

FUNGI IMPERFECTI
MELANCONIALES
MELANCONIACEAE

PHYTOPATHOGEN

Gen. Coryneum Nees

ICONOGRAPHIA MYCOLOGICA
Verona — Benedek
Plate A 3

Numerous species are found in North America. To a large extent the species are parasitic, causing the type of plant disease known as anthracnose. The genus Coryneum has numerous species. *C. beijerinckii* OUD. occurs in Europe and in various parts of the United States as the cause of a serious disease of the peach (*Amygdalus persica*). It kills twigs or may attack the buds and merely kill these and the surrounding tissues, or may cause injury to the fruits. The four or more celled spores are dark and long stalked. Conidia produced singly or in chains, often surrounded by a gummy mass, from conidiophores packed closely in a usually subepidermal or subcortical layer, the acervulus.

C. beijerinckii OUD.

a Acervulus
b Conidiophores with conidia

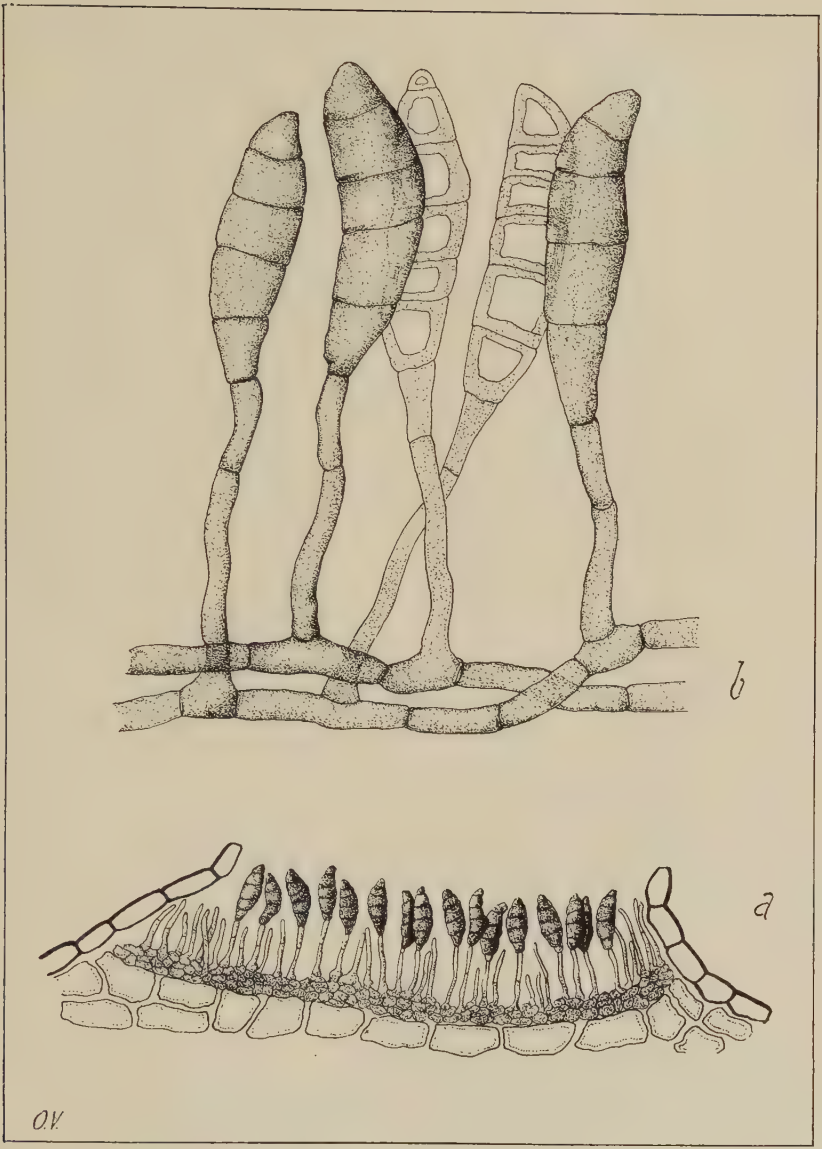


PLATE A 3

FUNGI IMPERFECTI
MONILIALES
DEMATIACEAE

SAPROPHYTA
PHYTOPATHOGEN

Gen. Helicosporium NEES.

Gen. Helicoma CDA

Gen. Helicomina OLIVE

ICONOGRAPHIA MYCOLOGICA
VERONA — BENEDEK
Plate A 4

Hyphae typically dark, olive to brown or black, rarely hyaline but the conidia then dark, loose and byssoid. Sterile and fertile hyphae or conidiophores both present as a rule.

Conidia spiral or convolute, cylindric, dark or hyaline, typically septate.

1 – Helicosporum NEES

Conidia relatively thick, not hygroscopic

2 – Helicoma CDA

Conidia relatively thin, hygroscopic

3 – Helicomina OLIVE

Genus near Helicoma, but phytopatogen

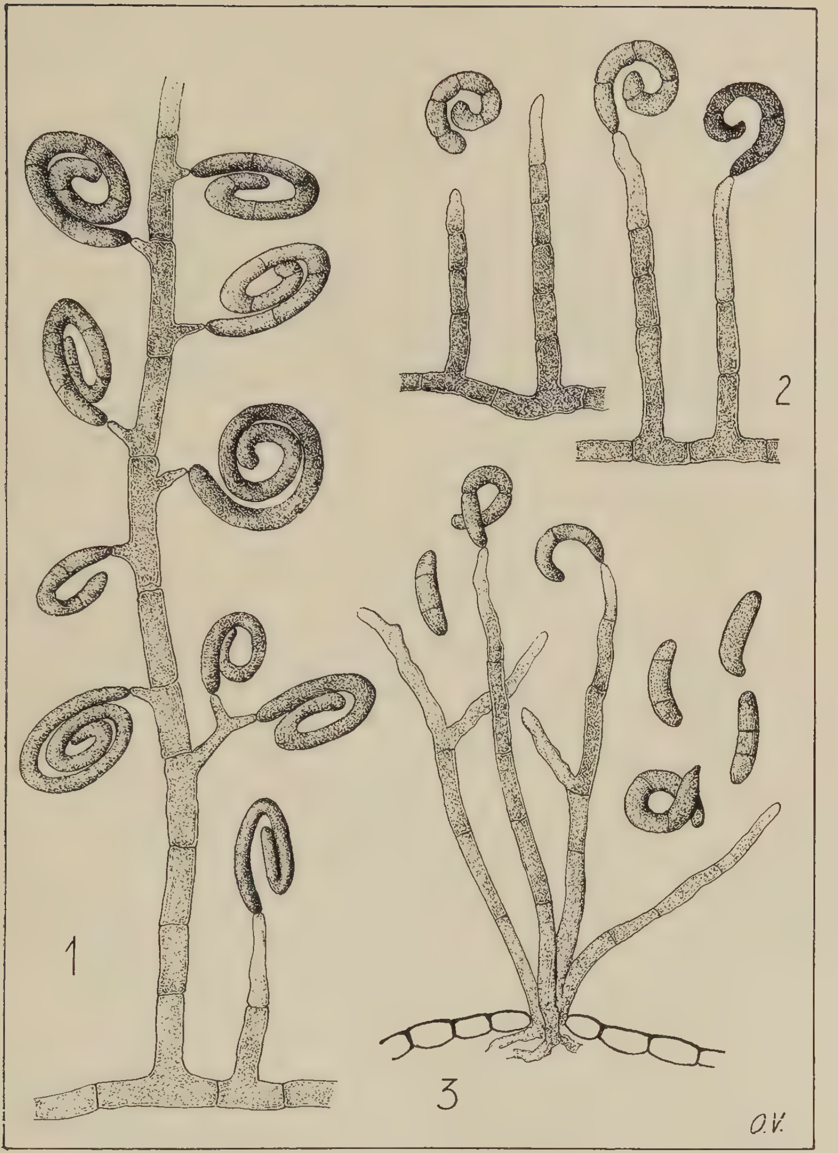


PLATE A 4

PHYCOMYCETES
MUCORALES
CEPHALIDACEAE

SAPROPHYTA

Gen. Linderina RAPER et FENNEL

Gen. Coemansia v. TIEGH. et LE MONNIER

ICONOGRAPHIA MYCOLOGICA

VERONA — BENEDEK

Plate B 1

1. Genus Linderina

Long, branched conidiophores with several sporocladia bearing on the upper surface phialides and hyaline, 1-celled elongated conidia.

2. Genus Coemansia

Slender conidiophora little branched with sporocladia bearing on the lower surface hyaline 1-celled ovoid to fusoid conidia.

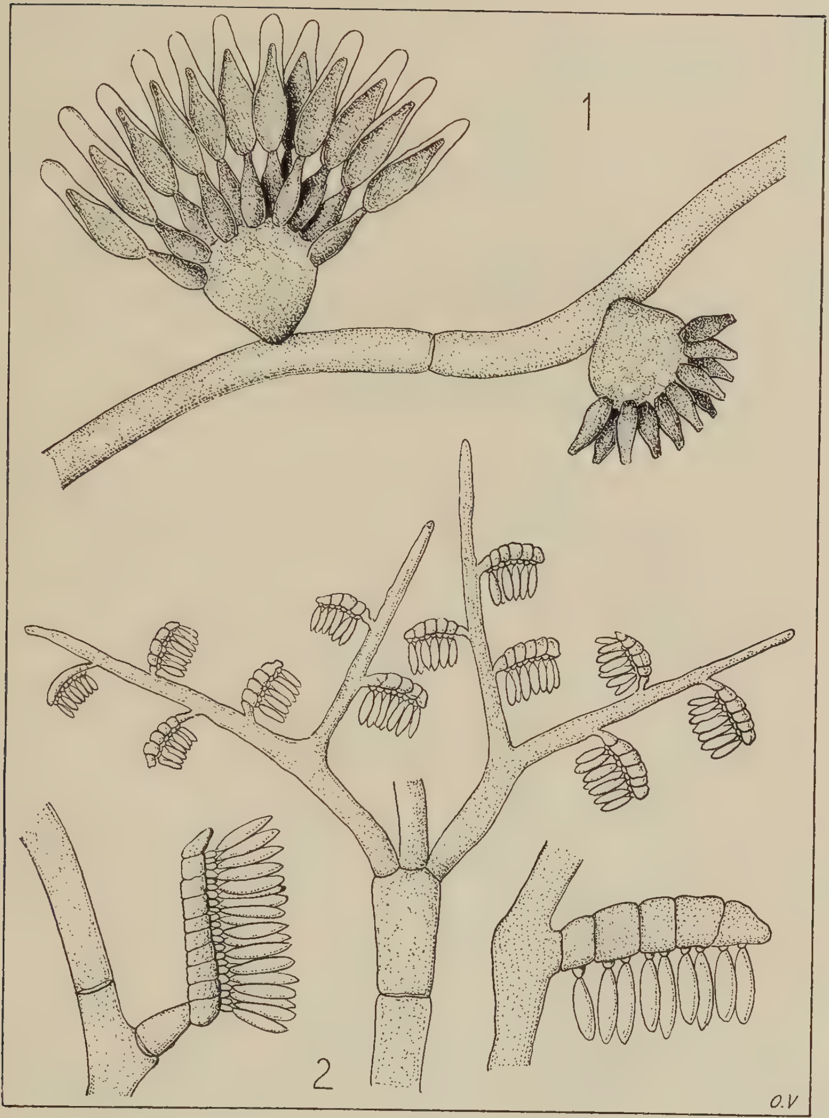


PLATE B 1

PHYCOMYCETES
BLASTOCLADIALES
BLASTOCLADIACEAE

SAPROPHYTA

Gen. Blastocladiella MATTHEWS

Gen. Blastocladia REINSCH

ICONOGRAPHIA MYCOLOGICA
VERONA — BENEDEK
Plate B 2

In Blastocladiella the basal cell is anchored by rhizoids and bears a single sporangium, a single gametangium or a single resting spore.

The thallus of Blastocladia consists of a relatively massive trunk, anchored in the substratum by rhizoids, and crowned by stout branches bearing zoosporangia and resting spores with pitted walls. They occur on submerged twigs and fruits, on animal material in the water, and in the soil. The thallus bears sessile zoosporangia liberating zoospores with one posterior flagellum, and round resting spores covered by densely pitted walls.

1 – Genus Blastocladiella

2 – Genus Blastocladia

a detail showing hair formation
b plant bearing sporangia and hypnospores
c zoospores

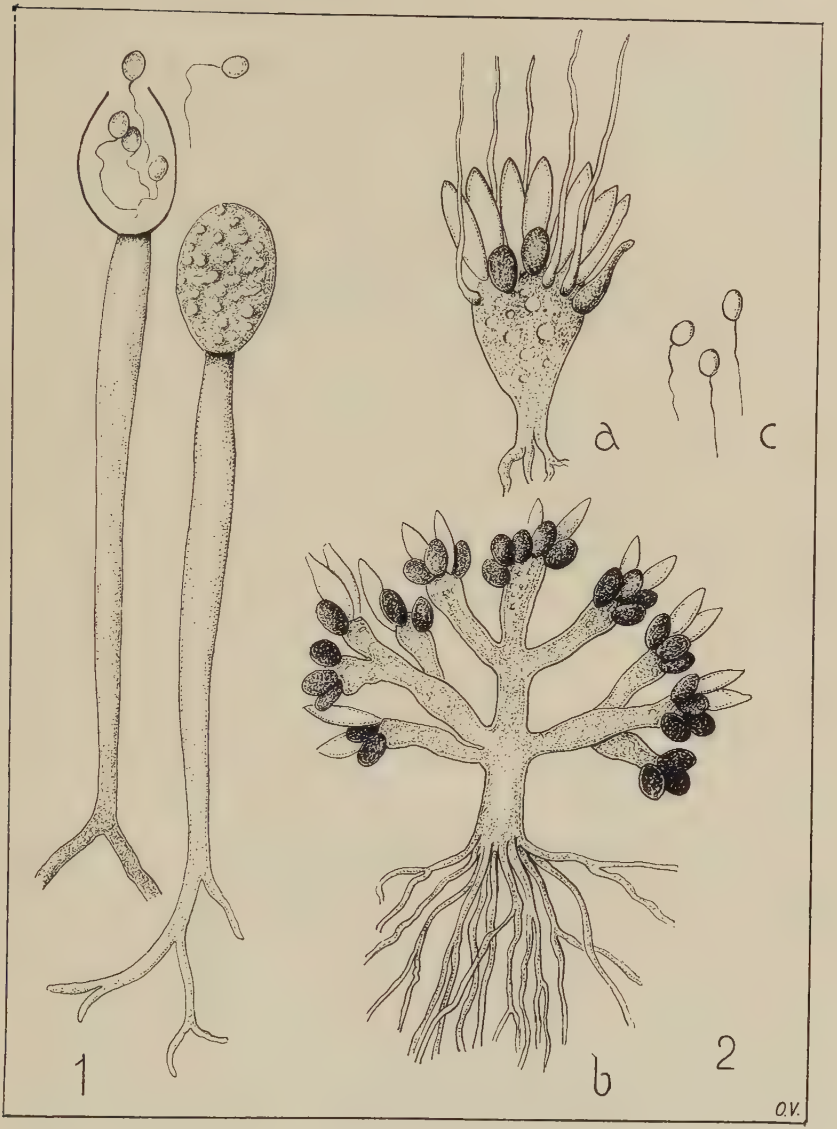


PLATE B 2

PHYCOMYCETES
MUCORALES
MUCORACEAE

SAPROPHYTA
(INDUSTRIAL)

Gen. Mucor MICHELI

ICONOGRAPHIA MYCOLOGICA

VERONA — BENEDEK

Plate B 3

Sporangia relatively large and many spored, oval or spherical, with a well developed columella. The sporangia are all alike and are borne singly, or several may be produced on a racemosely or otherwise branched sporangiophore. The zygospores are naked or more or less protected. Some species may have technical interest.

Species: *M. mucedo* (L.) BREFELD

1 – Sporangia
  a way of ramification of hyphae and sporangiophores
  b detail of sporangium with
  c columella
  s spores

2 – Formation of zygospores
  a, b, c different stages of development of the zygospore
  z zygospore

3 – budding mycelia with oidia (in anaerobiosis)

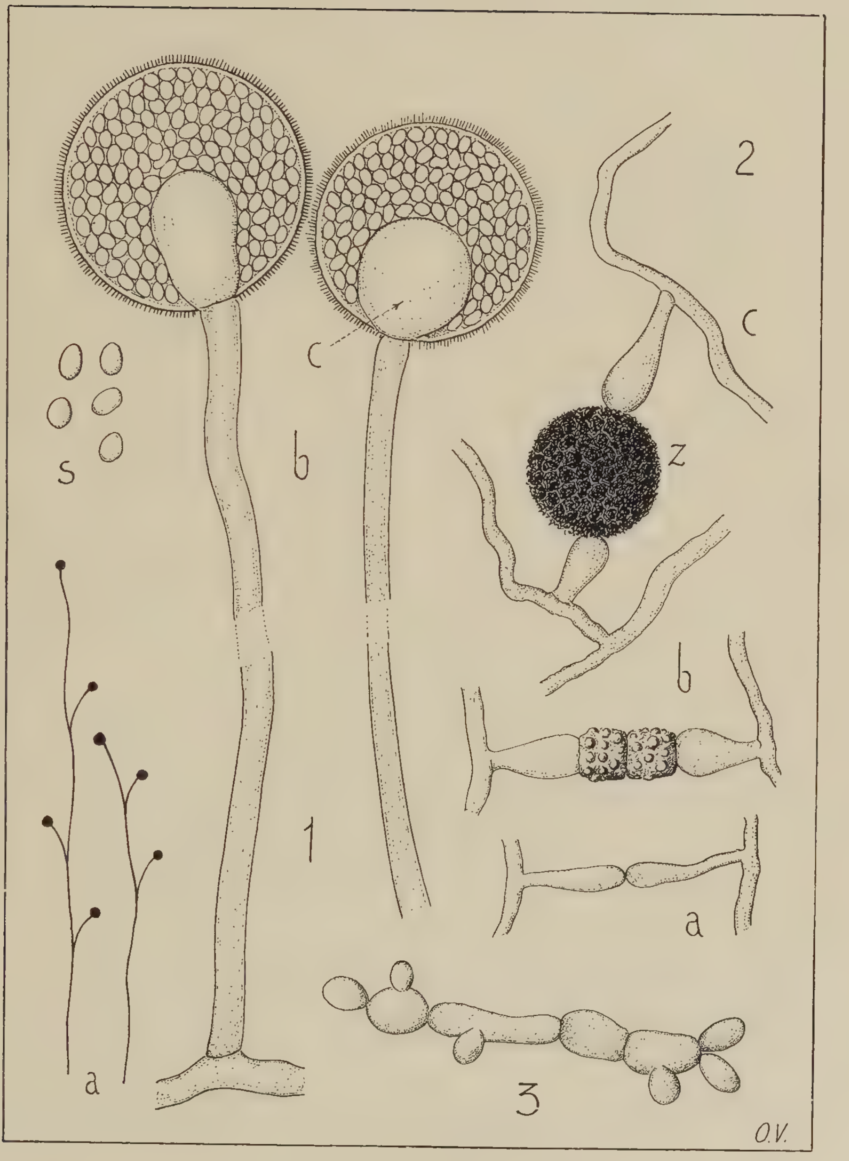

2
c
c
z
s
b
b
1
a
a
3
O.V.

PLATE B 3

PHYCOMYCETES
SAPROLEGNIALES
SAPROLEGNIACEAE

SAPROPHYTA

Gen. Brevilegnia COKER et COUCH

ICONOGRAPHIA MYCOLOGICA

VERONA — BENEDEK

Plate B 4

A true mycelium consists of non-septate, multinucleate coenocytic hyphae or of filaments with septa dividing them into uninucleate or binucleate cells or into short coenocytic segments. Only rarely is a mycelium unbranched. The hyphae of many of the aquatic forms are relatively large, occasionally attaining a diameter of over 100 microns. The nuclei are small and numerous. Asexual reproduction is by the formation of zoospores in terminal zoosporangia. In the unmodified zoosporangia these naked masses round up and develop 1 or 2 flagella and escape from an opening in the zoosporangium wall.

O	Oogone
S	Sporangium
Z	Zoospores

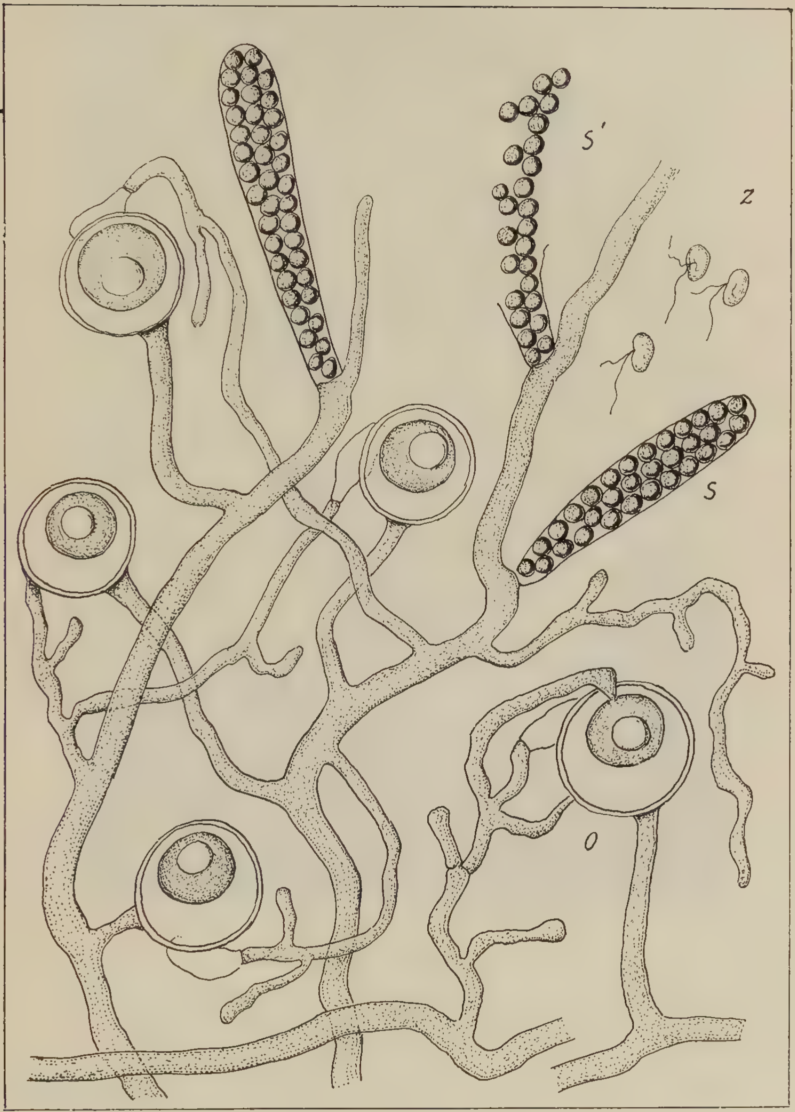


PLATE B 4

O. VERONA

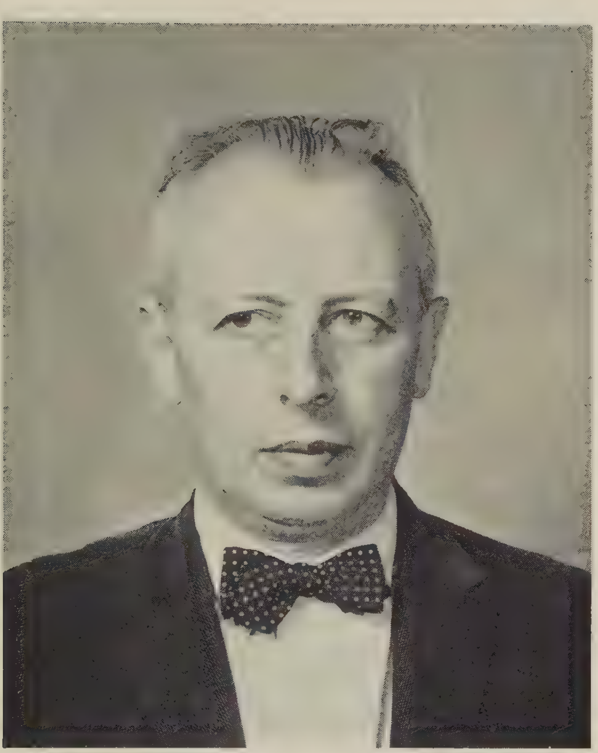
Istituto di Patologia Vegetale e Microbiologia Agraria e Tecnica
Università di Pisa, Italia

Gallery of Contemporary Noted Mycologists
45

G. A. DE VRIES

Centraalbureau voor Schimmelcultures
Baarn, The Netherlands

Gallery of Contemporary Noted Mycologists
46



J. SCHWARZ

Mycological Laboratory
The Jewish Hospital Association
Cincinnati, Ohio, U.S.A.

Gallery of Contemporary Noted Mycologists
47

J. A. NANNFELDT

Institute for Systematic Botany
University of Uppsala, Sweden

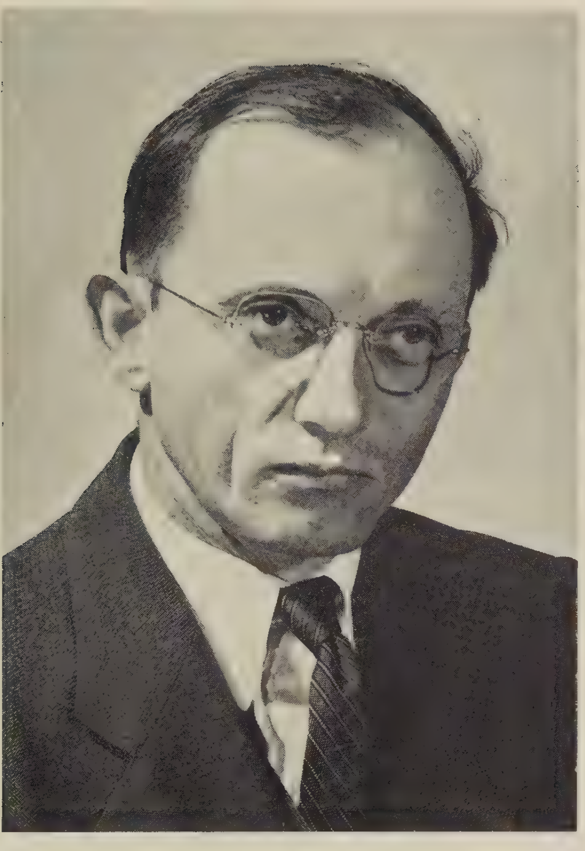
Gallery of Contemporary Noted Mycologists
48

S. SAKAGUCHI

Institute of Applied Microbiology
University of Tokyo, Japan

CARLOS DA SILVA LACAZ
Instituto de Microbiologia e Imunologia
Faculdade de Medicina da Universidade
São Paulo, Brazil

Gallery of Contemporary Noted Mycologists
51



ALEXANDER H. SMITH

Department of Botany
University of Michigan
Ann Arbor, Michigan, U.S.A.

Gallery of Contemporary Noted Mycologists
52

STUDIES ON THE NUCLEUS OF CANDIDA SPP.[1]

ABE WIDRA

(*Department of Bacteriology and Immunology, University of North Carolina School of Medicine, Chapel Hill*)

(6.VII.1959)

INTRODUCTION

Investigations on the nuclear cytology of Fungi Imperfecti have been relatively scarce. *Candida* spp. were selected for investigation because they 1) exhibit yeast-mold dimorphism, 2) may form ascospores, and 3) are sometimes pathogenic. This paper is a descriptive account of nuclear division in *Candida albicans* during budding and blastospore formation. Similar figures were found in *Candida tropicalis* and *Candida guilliermondi*. The normal sequence of nuclear events provides a baseline for studies on drug and radiation effects, morphogenesis, phylogeny, or ascosporogenesis.

The classic division figures seen in ascomycetes have been mainly the result of work with diploid conjugation nuclei (OLIVE, 1953). These figures are less often seen during division of the haploid nucleus. Inability to resolve intranuclear detail handicaps the investigator and this is the case with *Candida*. It may be that stained and squashed preparations will ultimately provide the solution for this problem. Meanwhile, the term ,,cryptomitosis'' has been proposed for those yeasts where the genetic evidence favoring Mendelian segregation of characters is not yet confirmed cytologically (HASHIMOTO et al., 1959). Since certain "imperfect" *Candida* species have demonstrated the wherewithal for mating and ascospore production when given the opportunity (LODDER & KREGER-VAN RIJ, 1952) the terms conventionally used in describing mitosis in ascomycetes appear here (ELLIOT, 1956).

MATERIALS AND METHODS

The chief organisms used were *C. albicans* strain 210, isolated in 1957 at the University of North Carolina from a foot lesion, and strain 412, isolated from sputum at Duke University in 1951.

---

[1] Supported by U.S.P.H.S. Grant E-1700.

Yeast phase cells were grown on Sabouraud's dextrose agar (DIFCO) at 37° C. Mold phase was obtained on corn meal agar (BBL) at 25° C. Colchicine, when used, was in 1 per cent final concentration in the above media.

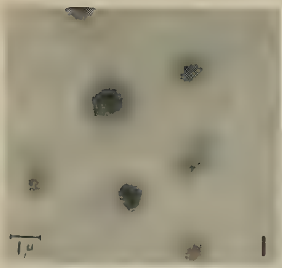
Fixing, staining, and dehydrating procedures were carried out in Columbia dishes. Impression smears on #0 coverslips were fixed in osmium tetroxide vapors for 1—2 minutes then plunged into ethanol packed in dry ice and held there for periods from 20 minutes to 2 hours. The smears were then rapidly run through a descending alcohol series to water before acid hydrolysis. This fixation procedure gave clean staining and produced minimal shrinkage (ENGSTROM & FINEAN, 1958).

Acid hydrolysis (N HCl at 60° C for 8 min) followed by the Azure A-thionyl chloride staining and dehydration method of DELAMATER (1948a) resulted in permanent mounts showing the nuclear apparatus of these organisms. Coverslips were mounted in HSR (Harleco Synthetic Resin). A time series of smears was run in order to help derive the proper sequence of divisional stages. All stages may be found in from 2—8 hours at 37° C after seeding.

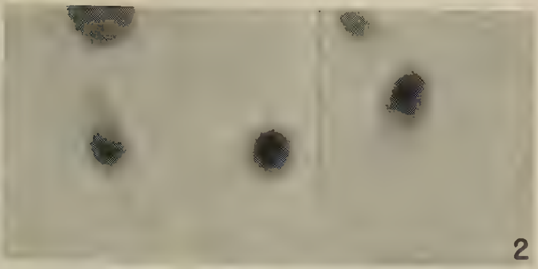
Photographs were taken through a 90 × (1.40 NA) apochromatic objective lens, matching oiled condenser, and a 15 × compensating eyepiece on 35 mm Kodak Microfile film. This primary magnification of 1350 × was increased slightly over 3 × in printing to give a final magnification of 5000 diameters. The scale in figure 1 applies to all the photographs. Figures 1—12 are of *C. albicans* strain 210; the remainder are of strain 412.

RESULTS

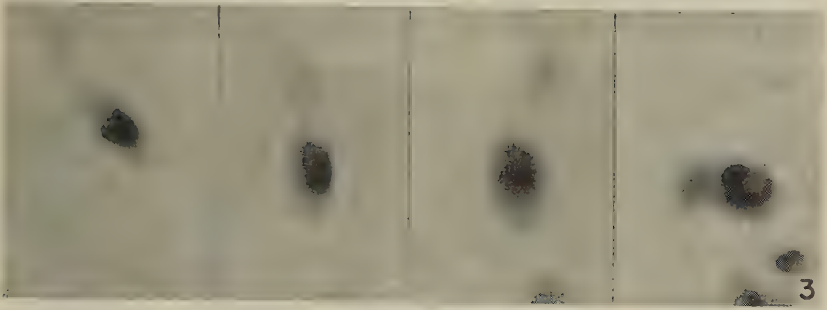
The interphase nucleus (figure 1) is typical of those which can be found in the blastospores of cells on corn meal agar culture 4—7 days old. It presents a semi-lunar condensed chromatinic mass polarized to a single granule (central body). This same orientation may be seen in *Blastomyces dermatitidis* (DELAMATER, 1948b; BAKERSPIGEL, 1957), *Saccharomyces cerevisiae* (DELAMATER, 1950), and *Schizosaccharomyces octosporus* (WIDRA & DELAMATER, 1955). During early prophase (figure 2), the cell enlarges and its chromatinic material swells up to fill the nuclear vesicle. This is more apparent during late prophase when a containing membrane seems to be present (figure 3). A deeper staining granule, presumably the central body, is sometimes still distinguishable at this time although in most cases it is absent from prophase cells. Figure 4 shows the metaphase "ring" stage: actually a tugrid sphere with the duplicated chromatin lying peripherally against the nuclear membrane. ECKSTEIN (1958) has described a similar metaphase "ring"stage during mitosis in a wild yeast. Figure 5 shows two levels of a pre-anaphase cell; in this instance, two almost separated masses lie within the nuclear vesicle. Various anaphase aspects are shown in figures 6 and 7. The meta-



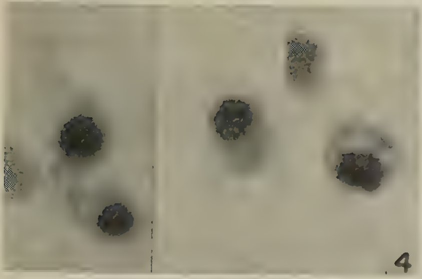
1



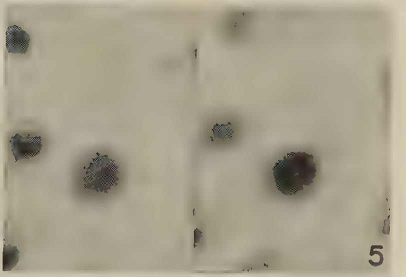
2



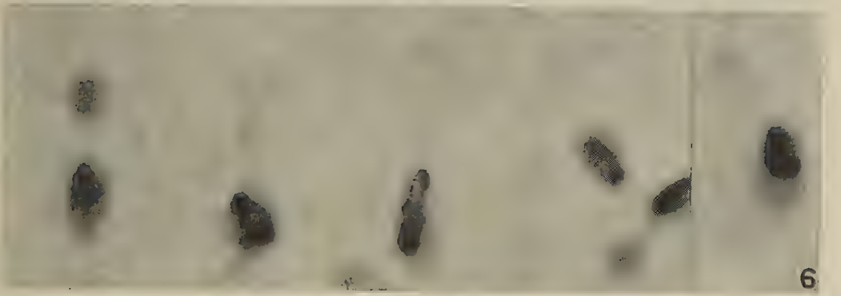
3



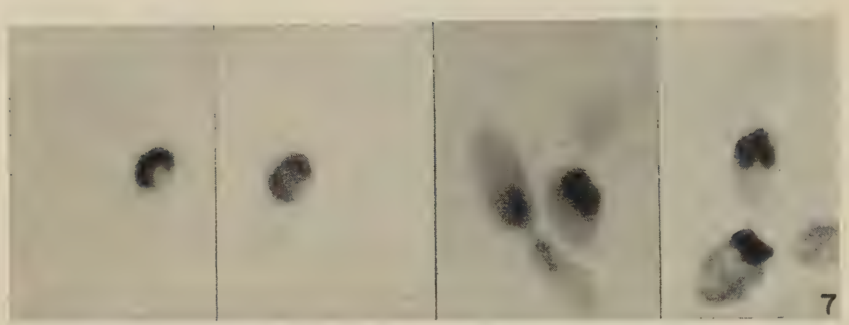
4



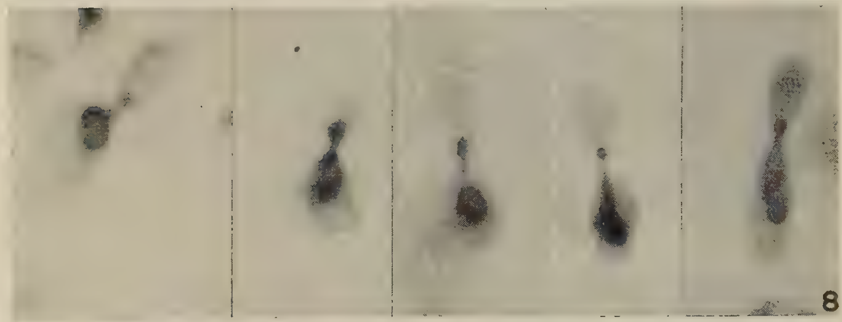
5



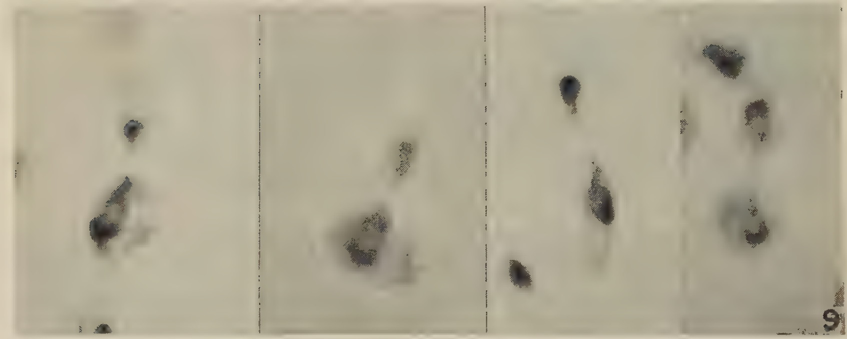
6



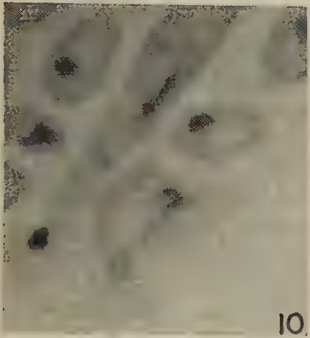
7

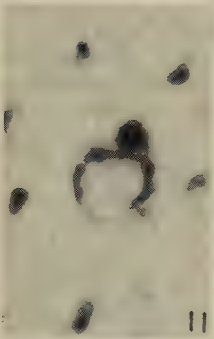


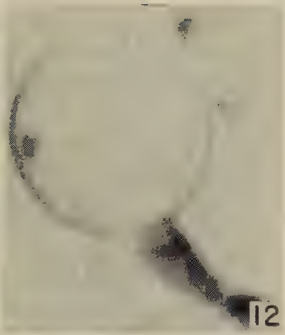
8



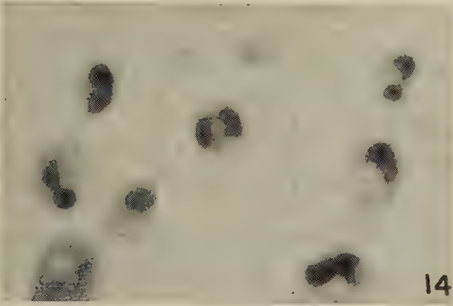
9

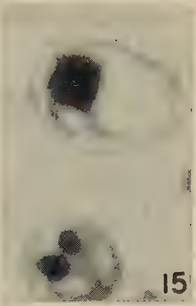

10

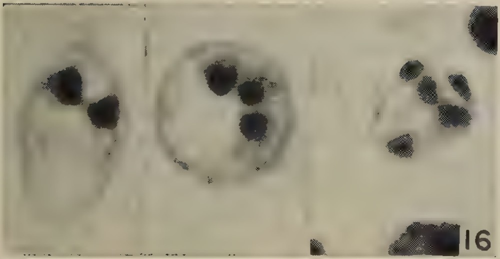

11

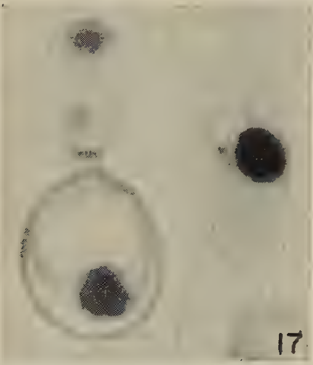

12

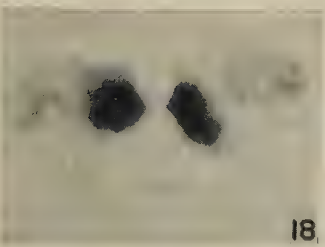
13

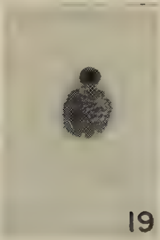

14

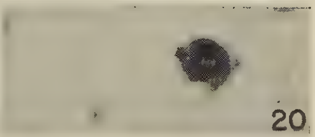

15


16


17


18


19


20

phase "ring" may open eccentrically to one side or at two points equationally across a diameter as anaphase movement begins. At first the axis of nuclear division is completely unoriented to the axis of cell division. Later an orienting force is apparent and the central body may again be observed during late anaphase (figure 8). A non-chromatinic area is often seen at the core of the sac-like nuclear vesicle at this point. The nuclear membrane breaks and the chromatin masses separate completely at telophase. Reorganization into the interphase aspects goes on in the mother and bud cells even as the last of the nuclear material is migrating through the bud canal (figure 9). No chromosomes could be resolved, but their presence must be inferred from the genetic data on *Saccharomyces* (CATCHESIDE, 1951)

Nuclear division in the mold phase duplicates that found in the yeast phase. In figure 10, migration of a daughter nucleus into a blastospore is seen. The large protochlamydospore in figure 11 illustrates the peri-vacuolar red-staining volutin characteristically seen in these cells. The nucleus, as usual, is stained blue. Figure 12 shows a mature thick-walled chlamydospore with the nucleus located to the left lying within a narrow strip of cytoplasm. The large central vacuole is loaded with fat.

Figures 13—20 illustrate *C. albicans* strain 412 on colchicine containing media. Only this strain would give polynucleate and polyploid giant cells.

Figure 13 illustrates the apparently normal prophase nucleus of a colchicine grown cell. After several days on colchicine agar, between 1 and 10 % of the cells examined will exhibit nuclear aberrations. Figure 14 illustrates intracellular anaphase stages of a binucleate series. Figures 15—18 illustrate various degrees of ploidy and giant cells capable of budding. The observation in figure 18 resulted when colchicine grown giant cells were streaked on plain Sabouraud's dextrose agar. Ploidy is "transient" (DELAMATER 1956) in these cells and disappears when colchicine is removed. Figure 19 shows the interphase nucleus of a giant cell and the associated prominent central body. In figure 20 a giant cell nucleus with two central bodies is seen.

DISCUSSION

The dark-staining nucleolus-like central body appears to function as a centriole (DELAMATER, 1948b; WIDRA & DELAMATER, 1955). This undifferentiated spherical granule, in *Schizosaccharomyces octospores* at least, is stainable throughout almost the entire division cycle during meiosis and mitosis. Furthermore this granule assumes a position external to the chromosomes during metaphase and is connected to them by means of a spindle. In *Candida* on the other hand, the central body disappears in late prophase and no spindle external to the dividing mass is seen.

It would seem that in *Candida* the central body and a spindle apparatus operate within the nuclear membrane, since a darker-staining granule is seen leading the chromatinic matrix into the daughter cell during anaphase. Until the identity of this darker-staining granule can be resolved, the term centriole is reserved for its classic use (SWANSON, 1957).

The central body or granule described in this paper must not be confused with the "centriole" of LINDEGREN and co-workers (1959). Most investigators of yeast nuclei agree 1) that LINDEGREN'S "centriole" is in reality the nucleus of the yeast cell and 2) that the yeast vacuole is not a part of the nuclear apparatus (WIDRA & DELAMATER, 1955; WINGE & ROBERTS, 1958; ECKSTEIN, 1958; HASHIMOTO et al., 1959).

Although an external spindle could not be observed, a spindle apparatus is inferred from the aberrations induced by colchicine (EIGSTI & DUSTIN, 1955). Anaphase separation of the sister nuclei during budding in *Candida* may be due to the pushing action of an internal spindle apparatus (CARLSON, 1956).

The central body of the yeast nucleus has been stained by a variety of methods involving acid hydrolysis followed by formaldehyde-basic fuchsin, Giemsa, or Azure A-thionyl chloride. These methods have demonstrated their equivalence to the Feulgen reaction in revealing DNA-containing structures. However, they are not as specific as the Feulgen in that excess basic dye is present. The Azure A-thionyl chloride method for example will stain the protein moiety in onion root tip nucleoli as well as the chromosomes. This is due to excess unreacted Azure A. The Feulgen reaction leaves these nucleoli unstained. Feulgen staining of the yeast nucleus central body has not been demonstrated by those using this technique (MARGOLENA, 1932; ROCHLIN, 1933; LIETZ, 1951; ECKSTEIN, 1958); its exact constitution remains a moot question. It is possible that the central body combines both nucleolar and centriolar functions. In haploid nuclei of *Sorosphaera veronicae* such functions are apparently combined in the nucleolus during "cruciform division", although the nucleolus disappears and an external spindle apparatus operates during the first meiotic division (MILLER, 1958). The "endosome" described by LEEDALE (1958) during mitosis in *Euglena* spp. contains RNA, is Feulgen negative, and resembles the nucleolus found in "cruciform division". Colchicine has no effect on mitosis in *Euglena*.

Apparently a classic spindle apparatus is not a necessary concommitant of mitosis in haploid microorganisms.

SUMMARY

Nuclear division in *Candida albicans* during budding and blastospore formation is described. Classic mitotic division as it occurs in the higher ascomycetes is not seen. Instead, the semi-lunar (crescentic) chromatinic material in the nucleus increases so as to fill the

nuclear vesicle, often assuming a toroid-like aspect. The nucleus then divides into two crescentic masses one of which migrates to the daughter cell. No external centriole or spindle apparatus could be observed. No chromosomes could be resolved. Nuclear division in the mold phase duplicated that found in the yeast phase. Colchicine treatment of yeast phase cells resulted in the appearance of large polynucleate and polyploid cells in one strain of *C. albicans*.

Bibliography

Bakerspigel, A. (1957) The structure and mode of division of the nuclei in the yeast cells and mycelium of *Blastomyces dermatitidis*; Canad. J. Microbiol., **3,** 923—936.

Carlson, J. G. (1956) On the mitotic movements of chromosomes; Science, **124,** 203—206.

Catcheside, D. G. (1951) The Genetics of Microorganisms; Pitman & Sons, London, 110—114.

DeLamater, E. D. (1948a) Basic fuchsin as a nuclear stain for fungi; Mycologia, **40,** 423—429.

DeLamater, E. D. (1948b) The nuclear cytology of *Blastomyces dermatitidis*; Mycologia, **40,** 430—444.

DeLamater, E. D. (1950) The nuclear cytology of the vegetative diplophase of *Saccharomyces cerevisiae*; J. Bact., **60,** 321—332.

DeLamater, E. D. (1956) Bacterial chromosomes and their mechanism of division in Bacterial Anatomy; University Press, Cambridge, 215—260.

Eckstein, B. (1958) Karyologische Untersuchungen an einer Wildhefe; Arch. Mikrobiol., **32,** 65—80.

Eigsti, O. J. & Dustin, P. (1955) Colchicine in Agriculture, Medicine, Biology and Chemistry; Iowa State College Press, Ames.

Elliot, C. G. (1956) Chromosomes in microorganisms in Bacterial Anatomy; University Press, Cambridge, 279—295.

Engstrom, A. & Finean, J. B. (1958) Biological Ultrastructure; Academic Press, New York.

Hashimoto, T., Conti, S. F. & Naylor, S. B. (1959) Studies on the fine structure of microorganisms; J. Bact., **77,** 344—354.

Leedale, G. F. (1958) Nuclear structure and mitosis in Euglenineae; Arch. Mikrobiol., **32,** 32—64.

Lietz, K. (1951) Beitrag zur Hefecytologie; Arch. Mikrobiol., **16,** 275—302.

Lindegren C. C., Pittman, D. D. & Yuasa, A. (1959) The centriole as the primary radiation target in the resting cell; Trans. N. Y. Acad. Science, **21,** 524—530.

Lodder, J. & Kreger-van Rij, N. J. W. (1952) The Yeasts: A Taxonomic Study; North Holland Pub., Amsterdam.

Margolena, L. A. (1932) Feulgen's reaction and some of its applications for botanical material; Stain Tech., **7,** 9—16.

Miller, C. E. (1958) Morphology and cytology of the zoosporangia and cystosori of Sorosphaera veronicae; J. Elisha Mitchell Scien. Soc., **74,** 49—64.

Olive, L. S. (1953) The structure and behavior of fungus nuclei; Bot. Rev., **19,** 439—586.

Rochlin, E. (1933) Über die Nuklealreaktion bei Hefen; Zbl. Bakt. Parasitenk., Abt. II, **88,** 304—306.

Swanson, C. P. (1957) Cytology and Cytogenetics; Prentice-Hall, Englewood Cliffs, N. J., 46—62.

Widra, A. & DeLamater, E. D. (1955) The cytology of meiosis in *Schizosaccharomyces octosporus*; Amer. J. Bot., **42,** 423—435.

Winge, O. & Roberts, C. (1958) Life history and cytology of yeasts; in The Chemistry and Biology of Yeasts; Academic Press, New York.

STUDIES ON THE RESPIRATION OF *TRICHOPHYTON RUBRUM* [1]

E. E. Vicher, I. Lyon [2] & E. L. White

(*Department of Microbiology, University of Illinois College of Medicine, Chicago, and Biological Department, Research Laboratories, The Toni Company, Chicago, Illinois*).

(14.II.1959)

One of the principal difficulties in evaluating chemotherapeutic agents is that of establishing adequate criteria for their activity. These criteria are usually determined by bioassay techniques which are (a) qualitative, i.e. those in which an event such as growth does or does not occur and/or (b) quantitative, i.e. those which provide a measure of the effect of some agent upon an organism (Golden & Oster, (1950); McCallan & Wilcoxon (1934), (1939), and (1940); Oster & Golden, (1947)). The former group of techniques is based upon the observation of a given "response phenomenon" and is in common use today. For the evaluation of potential antifungal agents there is a need for the development of assay methods in which a "response phenomenon" is elicited under *in vitro* conditions approximating those found *in vivo* for a particular fungus. It was hoped that these studies would provide a suitable "response phenomenon" assay and, in addition, a method for the investigation of some of the fundamental aspects of fungal metabolism.

In an attempt to develop such methods workers have undertaken the study of oxygen uptake by *Trichophyton rubrum*, *T. gypseum*, *Epidermophyton floccossum* and *Microsporum canis*. Such studies have been carried out with variations in carbon source, salt concentration, pH of incubation medium, etc. The effects upon respiration of the fungus under study were determined for various inorganic salts, organic acids, and certain other organic compounds (McCallan & Wilcoxon, (1934) and (1939)).

In the studies reported here techniques previously employed by others have been modified for respiration experiments with *T. rubrum*. Oxygen uptake (Q_{O_2}) levels have been correlated with the morphology and the age of the fungus. The effects on fungal respiration of the presence or absence of glucose in the growth medium and of variations in salt composition and concentration in the incubation medium have been assessed. An attempt has been made to correlate some of the *in vitro* respiration data with the antifungal activity of some compounds now used in the clinical treatment of dermatophyte infections.

---

[1] This investigation was supported by a research grant from the Toni Company, Chicago, Illinois.

[2] Present address: Department of Biological Chemistry, University of Illinois, College of Medicine, Chicago, Illinois.

MATERIALS AND METHODS

Preparation of the fungus. *Trichophyton rubrum* was grown on the medium used by NICKERSON & CHADWICK (1946). A sterile circle of Whatman No. 40 filter paper, 7 cm in diameter, was placed upon the moist surface of freshly prepared growth medium. Intimate contact of all of the paper with the surface of the medium was effected.

A homogenate of *T. rubrum* was prepared by washing a Sabouraud's agar slant growth, one week old, into a small stainless steel cylinder (Sorvall Omni-Mixer) with 20 ml phosphate buffer at pH 6.0. Homogenization was carried out at 9000 rpm for one minute. A sample of the homogenate (0.2 ml) was seeded onto the center of the filter paper on the growth medium as described above. This inoculum was spread over the entire surface with a sterile wire spreader, the dish was sealed with one inch adhesive tape, and then placed into the incubator at 28° C.

After the incubation period the filter paper, upon which the fungus mat had grown, was transferred aseptically to a sterile Petri plate and washed with sterile distilled water for 24 hours to remove excess nutrients. This procedure was followed because preliminary experiments indicated that the organism so treated, approximating a ,,starved" organism, exhibited a higher respiratory activity upon subsequent incubation. These results would appear to preclude the probability of removal of essential nutrients. The washed mat was removed from the Petri dish and cut into 15 mm disks with a sterile cork borer.

Preparation of the media. The growth medium consisted of glucose, 50 g; tryptose, 10 g; asparagine, 1 g; yeast extract (Difco), 0.1 g; K_2HPO_4, 5 g; $MgSO_4$, 0.20 g; agar, 15 g; and distilled water, 1000 ml. The above ingredients, except glucose, were dissolved in 900 ml distilled water, titrated with HCl to a pH of 6.0, and autoclaved at 121° C for 20 minutes. A sterile 50 % aqueous glucose solution (100 ml) was added to the melted agar (50 C). After thorough agitation 20 ml of the medium were poured into each of 50 sterile Petri plates and allowed to congeal.

The incubation medium contained the following: glucose, 50 g; tryptose, 10 g; asparagine, 1 g; yeast extract (Difco), 0.01 g; KH_2PO_4, 0.07 g; $MgSO_4$, 0.07 g; $Al_2(SO_4)_3$, 0.90 g; NaCl, 2.50 g; KCl, 0.30 g; $CaCl_2$, 0.06 g; $NaHCO_3$, 1.20 g; and distilled water, 1000 ml. All ingredients, except glucose, were dissolved in 900 ml distilled water, titrated with HCl to pH 6.0, and autoclaved at

121° C for 20 minutes. Upon cooling, 100 ml sterile aqueous glucose solution were added. This bicarbonate-buffered medium was stored at 4° C in 100 ml portions, each in a 250 ml Erlenmeyer flask sealed with parafilm, until ready to use.

Respiration studies. Two types of experiments were performed. In the respiration studies, 2.6 ml of the incubation medium were added to the main compartment of each Warburg flask into which five 15 mm disks were placed. The center well contained 0.4 ml of a 40 % KOH solution and a thin strip of filter paper to increase the absorptive surface of the base for trapping evolved CO_2. Thus, the total fluid volume of each flask was 3.0 ml.

In the studies of the effects of various potential antifungal agents upon the $Q_{O_2}^{Air}$ of *T. rubrum* the main compartment contained 1.0 ml of the incubation medium. The total fluid volume within each flask was 3.0 ml.

The agents under study (in 1 ml of vehicle) were tipped into the main compartment at the start of incubation following a 15-minute equilibration period at 28° C. At that time the contents of the second sidearm (0.6 ml of the incubation medium) were also tipped into the main compartment. Readings were then recorded at 10-minute intervals for the next three hours. The oxygen consumed in each 10-minute interval was calculated. Final respiratory data are denoted by $Q_{O_2}^{Air}$ values (microliters of oxygen consumed in an atmosphere of air per hour per milligram dry weight of fungus tissue).

Morphological studies. The *T. rubrum* mats used in the respiration studies were examined to determine their gross and microscopic morphology.

Results

Influence of growth medium on morphology of T. rubrum. Primary cultures of *T. rubrum* develop numerous microconidia in clusters and singly along the hyphae. Few macroconidia, arthrospores, racket hyphae, or nodular bodies were observed. In skin and nails only hyphae and arthrospores are seen (Conant, *et al,* (1954)).

Routine examination of all mats (subcultures) was made. The mats were predominantly hyphal (about 95 %) with occasional arthrospores and nodular bodies (5 %). No changes in percentage composition of the mats at various age periods were noted.

Effect of mat mass on $Q_{O_2}^{Air}$. The respiratory data obtained with seven day old *T. rubrum* subcultures revealed a linear relationship between the logarithm of $Q_{O_2}^{Air}$ and the logarithm of milligrams of mat dry weight incubated. This relationship is clearly indicated on the next page in figure 1. The slope and y-intercept of the line in the figure were determined by the method of least squares.

$Q_{O_2}^{Air}$ correction factors (QCF)[3] were calculated from the analysis of the $Q_{O_2}^{Air}$-dry weight relationship. The QCF values have been used to correct all $Q_{O_2}^{Air}$ data, designated $Q_{O_2}^{Air}$ (corrected), which would have been obtained had a standard mat dry weight been incubated. For convenience the standard mat dry weight has been set at 14 milligrams (QCF = 1.000).

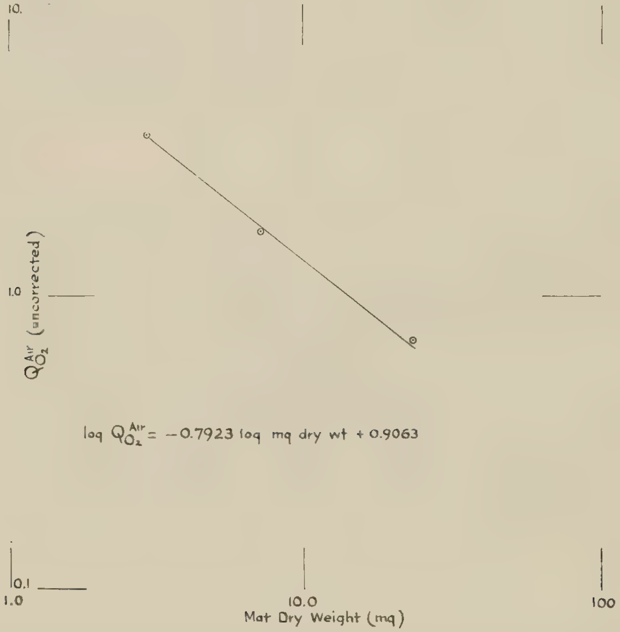


Figure 1. Effect of Mat Mass on $Q_{O_2}^{Air}$. The data were obtained with *T. rubrum* mycelia, seven days old, cultured on a glucose-containing growth medium.

Correlation of $Q_{O_2}^{Air}$ with age of organism. *T. rubrum* cultured on the described growth medium exhibited considerable variation in $Q_{O_2}^{Air}$ values with increasing age of the fungus. Table I shows the results obtained with (a) the organism cultured on a glucose-containing growth medium (two series of experiments) and (b) the fungus cultured on a glucose-free growth medium (one series of experiments). Subsequent incubations were carried out in the medium described above. The $Q_{O_2}^{Air}$ data were corrected for the effect of mat weight on Q_{O_2} and then averaged.

[3] $QCF_x = \frac{Q_{O_2} \text{ for 14 mg dry weight}}{Q_{O_2} \text{ for x mg dry weight}}$.

It may be assumed from the relationship between the age of the organism and the $Q_{O_2}^{Air}$ values that the time at which the first Q_{O_2} determination can be made is dependent upon the growth rate of the mycelial mat. That is, there must be a minimum number of mycelia present to reliably determine a Q_{O_2} value.

TABLE I.

Relationships among (a) age of organism, (b) respiratory activity, and (c) mat dry weight of T. rubrum cultured in the presence and in the absence of glucose

Age of Organism (days)	$Q_{O_2}^{Air}$ (corrected)	Mat Dry Weight (mg)
(a)	(b)	(c)
A. *Cultured on Glucose-Containing Growth Medium*		
2	3.3	—
3	11.5	15.3
4	12.3	—
5	8.9	15.3
6	6.9	17.7
7	6.3	20.1
8	5.0	20.7
9	4.8	21.3
10	3.9	34.6
11	3.6	47.9
12	2.9	58.2
13	2.6	68.5
14	2.0	74.4
15	1.8	80.4
17	1.9	76.3
19	1.8	66.6
21	2.3	—
22	2.2	—
23	1.8	—
25	1.9	—
B. *Cultured on Glucose-Free Growth Medium*		
5	7.7	22.5
7	5.7	26.0
9	4.3	19.0
11	3.5	29.0
13	3.0	26.2
15	2.7	22.0
17	2.6	18.5

Microrespiration effects of some antifungals. The effects of various antifungal agents upon the uptake of oxygen by *T. rubrum* are indicated in table II.

TABLE II.

Inhibition or stimulation of respiratory activity of T. rubrum by various antifungal agents added in vitro

Agent	Concentration (millimoles/liter)	Inhibition * (percent)	Stimulation * (percent)
A. *Alkyl Esters of p-Hydroxybenzoic Acid* **			
Methyl	21	45	
Propyl	18.5	37	
Butyl	17.1	48	
B. *Inorganic Salts* ***			
$AgNO_3$	196	41	
	60	8	
KCN	51.2	30	
	5.1		28
	0.51		17
H_3BO_3	269		3
	161		43
$KMnO_4$	2.1		23
	1.05		12
$Al(OOCCH_3)_3$	342		7
	171		2
KI	200	15	
	60.2		19

* Percent inhibition or stimulation is relative to the $Q_{O_2}^{Air}$ value obtained in the absence of the added agent (control).

** In an emulsion composed of 1.2 % cholesterol in 95 % ethanol. Inhibition due to the concentration of emulsion used per flask was 39 %.

*** In aqueous solution.

DISCUSSION

Preparation of the fungus. The effect of nutrients, antimetabolites, pH, and other factors upon microbial metabolism can be properly determined only in synchronous cultures. Such cultures display a high degree of uniformity in morphological characteristics and, presumably, also in metabolic activity. With *T. rubrum* it was not necessary to employ special techniques to induce synchrony since the cultures grown on the glucose-containing medium showed that over 95 $^0/_0$ of the organisms were in the form of mycelia.

NICKERSON (1946) and NICKERSON & CHADWICK (1946) grew the organism directly on an agar medium necessitating the removal of the medium by immersion of the mat into boiling water at the conclusion of a particular study. The agar retained with the fungus probably contained nutrient substances which were in contact with the organism during the subsequent Warburg incubation. Following incubation the fungus was dried and weighed. The filter paper technique described herein (a) permits the handling of the organism in a simple, reproducible manner before and following the Warburg incubation, and (b) facilitates transfer of the fungus into and out of the Warburg vessels. Further, this technique allows for the easy removal of all nutrients present in the medium during the growth period of the fungus. The filter paper mat technique is particularly advantageous in those studies, like our own, in which the growth and the incubation media differ in composition.

Morphology of the fungus. As described above there are some differences in the morphologically distinct forms of *T. rubrum* present (a) *in vivo* in skin and in nails (CONANT, *et al*, 1954)), (b) in primary cultures and (c) in our mat subcultures. In (a) hyphae and arthrospores predominate. In (b) there are numerous microconidia with few macroconidia, racket hyphae, arthrospores, and nodular bodies. The mat subcultures, (c) are primarily hyphal with occasional arthrospores and nodular bodies. Thus, the morphological characteristics of the mat subcultures are somewhat similar to those found *in vivo*.

Influence of incubation medium on $Q_{O_2}^{Air}$. No significant differences were noted in the respiratory activity of 14- to 21-day old *T. rubrum* subcultures when incubated in different media. In these studies, however, the bicarbonate-buffered incubation medium described above was used.

The concentration of K^+ ion was decreased markedly in comparison with the Na^+ ion concentration. It has been shown in *in vitro* studies with mammalian liver that a high K^+ ion concentration favors synthetic pathways while a high Na^+ ion concentration favors oxidative metabolism (HASTINGS, *et al*, (1952); LYON & GEYER, (1954); LYON, *et al*, (1955)).

It has been demonstrated that Al^{3+} catalyzes the non-enzymatic decarboxylation of oxaloacetic acid (KREBS & EGGLESTON, (1945)). In other studies, investigators have found that Al^{3+} and Ca^{2+} activate both succinoxidase (succinic dehydrogenase, cytochrome c, cytochrome oxidase, and the other components necessary for the transfer of hydrogen from succinate to oxygen) and ascorbic acid oxidase (HORECKER, *et al*, (1939); FRIEDEN & MAGGIOLO, (1957)). Furthermore, the oxidation of reduced diphosphopyridine nucleotide is accelerated by the presence of ascorbate and ascorbic acid oxidase (KERN & RACKER, (1954)). Thus, it appeared that the metabolism of endogenous C_4 dicarboxylic acids might be significantly stimulated by the addition of aluminium and calcium ions.

Effect of mat mass on $Q_{O_2}^{Air}$. The relationship between $Q_{O_2}^{Air}$ and dry weight of the fungal mat noted above in the results may be due to a diminishing surface/volume ratio with the increasing age of the organism. This relationship might be further dependent upon a differential rate between the rapid increase in cell volume and the slower increase in mitochondrial (major fraction of "functional") mass. The slower increase in "functional" mass may actually reflect a time lag in its rate of replication in comparison with the rate of cytoplasmic replication.

Correlation of $Q_{O_2}^{Air}$ with age of organism. Two significant characteristics were exhibited by the organism cultured on glucose (table I, A). (1) The highest $Q_{O_2}^{Air}$ values were obtained with organisms which were from three to five days old. This peak in respiratory activity occurred during a period of rapid growth. The mycelial mats had an average dry weight of 15.3 milligrams. (2) The $Q_{O_2}^{Air}$'s fell to minimal values at about 10 to 14 days of age (56.7 mg average dry weight) and remained at the minimal level up to 25 days of age. The mats of the 14- to 25-day old organisms had an average dry weight of 74.5 milligrams.

Five- to 17-day old *T. rubrum* organisms cultured on a glucose-free medium (table I, B) showed no apparent difference in respiratory activity from that seen in organisms of similar ages cultured on a glucose-containing medium. The mat dry weights, however, were considerably different. At five days mats weighed 22.5 mg, at 10 to 14 days they averaged 24.7 mg, and beyond 14 days the mat dry weights averaged 20.3 milligrams.

An analysis of the data in table I, A indicates three major processes. (1) During the first three to five days the increase in mass is paralleled by an increase in "functional mass", i.e. the total weight of metabolically active organelles within the living organism. (2) Beyond this early period, up to 10 to 14 days, continued growth apparently results in an increase in total mass without any further increase in "functional mass". This process exerts a dilution effect upon the $Q_{O_2}^{Air}$. (3) In organisms older than 14 days an equilibrium is evident between the $Q_{O_2}^{Air}$, a reflection of the "functional mass", and the total mass which remains at a fairly steady level.

The five- to 17-day old organisms cultured in the absence of glucose showed no measurable weight gain (table I, B). However, their mat dry weights for the period from three to 10 days, were approximately the same as those of the fungi cultured on glucose. Beyond 10 days the glucose-grown fungi gained markedly in mat dry weight indicating a significant net incorporation of glucose carbon atoms into fungal tissue. However, the respiratory efficiency of the organisms cultured in the absence of glucose was about three times that of the fungi grown in the presence of glucose, i.e., the former organisms, with one-third the tissue weight, exhibited the same respiratory activity as the latter organisms.

The relationships discussed above indicate that in *T. rubrum* subcultures an increase in mat weight is not a reliable index of respiratory activity. It may be reasonably assumed that the $Q_{O_2}^{Air}$ is primarily dependent upon the metabolically active organelles within the organisms. Thus, the increase in total mat dry weight seen in the glucose-cultured organism was probably due to incorporation of glucose carbon atoms into the metabolically inert structures (those with a low turnover rate?) within the fungal cell.

Microrespiration effects of some antifungals. (1) Fatty acids (free): — in the concentration range studied (50 to 500 millimoles per liter), propionic, octanoic, nonanoic, and undecylenic acids all depressed the respiration of *T. rubrum* approximately 90 %. It may be of interest to note that propionic acid is a three-carbon (odd) acid while octanoic acid has eight atoms (even). These acids are formed and oxidized by microorganisms via different metabolic routes (FOSTER, (1949)). Since the antifungal activities of these fatty acids are similar while their metabolism is different, it is suggested that their action may be non-metabolic. Perhaps these acids disrupt the cell wall of the fungus or denature fungal protein by a mechanism involving surface activity.

(2) Fatty acids (sodium salts): — Propionate, the saturated short chain fatty acid salt, appears to be less effective in depressing respiratory activity than the salt of a longer chain unsaturated fatty acid (undecylenate).

The undecylenate effect was independent of concentration in the 10-fold range from 16.1 to 161 millimoles per liter. The maximal propionate effect, about one-half of that due to undecylenate, was evident only at the higher concentration (351 millimoles per liter).

With the exception of propionate, the free fatty acids and their sodium salts caused an inhibition of respiration of 90 % or more in the 10-fold concentration range from 50 to 500 millimoles per liter. The apparent lack of effectiveness of propionate has led to further studies, now in progress, of the effects of pH and the concentration of these fatty acids on the respiratory activity of the fungus.

(3) Alkyl esters of p-hydroxybenzoic acid: — The inhibitory activities of the methyl, propyl, and butyl esters were approximately the same (table II, A), and not significantly higher than the inhibitory effect of the emulsion vehicle alone (39 % inhibition). These compounds are of interest because of their possible interposition between Krebs cycle intermediates and complex ring structures (FOSTER, (1949)).

(4) Inorganic salts: — Among these agents tested for antifungal effectiveness only $AgNO_3$ and KCN showed any significant inhibitory activity (table II, B). Silver ion is known generally as an effective protein denaturant and this action may account for its inhibition of respiratory activity. The effect of KCN is particularly striking since, at a concentration of 5.1 millimoles per liter, it stimulated respiration 28 % while at a concentration of 51.2 millimoles per liter it depressed respiration 30 %. These effects of KCN may be explained by the action of CN^- ion on the copper-containing enzyme ascorbic acid oxidase. It has been observed that low concentrations of CN^- activate while high concentrations inhibit the enzyme (FRIEDEN & MAGGIOLO, (1957)). Activation of the oxidase by the lower concentration of KCN could account for the resultant increase in respiratory activity. Conversely, the

higher concentration of KCN may have provided a sufficiently high CN^- ion concentration to inactivate the enzyme by chelating its copper. The interposition of the ascorbic acid oxidase system between diphosphopyridine nucleotide and flavoprotein in the respiratory electron transport chain provides for the oxidation of metabolites by alternative pathways, one sensitive to and the other insensitive to the effects of CN^- ion. The responses of *T. rubrum* to low and to high KCN concentrations suggests that the fungus possesses such alternative metabolic pathways.

Summary

The morphological characteristics of *Trichophyton rubrum* culture, on a glucose-containing medium are similar to those seen in infected skin and nails. The subcultures are primarily mycelial and show a high degree of synchrony.

The respiratory activity of the fungus is very high in the incubation medium developed and used in these studies.

Although *T. rubrum* grows well in the presence of glucose the fungus does not appear to be dependent upon glucose as a primary carbon source.

T. rubrum demonstrates a higher respiratory efficiency following growth in the absence of glucose than in its presence.

The $Q_{O_2}^{Air}$ of the fungus is markedly affected by the amount of fungal tissue incubated. This observation made it necessary to determine the relationship between the $Q_{O_2}^{Air}$ and the mat dry weight. This relationship may be expressed as $y = -0.7923\,x + 0.9063$, where y is the log $Q_{O_2}^{Air}$ and x is the log milligrams dry weight.

The $Q_{O_2}^{Air}$ of *T. rubrum* appears to be a reflection of the "functional mass" of the organism, i.e. the mass of the metabolically active organelles (mitochondria, microsomes, etc.). The highest respiratory activity was exhibited by subcultures three to five days old. In younger subcultures $Q_{O_2}^{Air}$ values were much lower. In older organisms respiratory activity declined to a basal level at 10 to 14 days and remained at that level throughout the period up to 25 to 28 days. Subcultures were not maintained beyond this time.

The following materials, of those tested, show a significant inhibitory effect on the respiration of *T. rubrum*: propionic, octanoic, nonanoic, and undecylenic acids, and sodium undecylenate.

$AgNO_3$ and a high concentration of KCN both caused an inhibition in the respiratory activity of the fungus. At a low concentration KCN stimulated respiration in the organism. These KCN effects suggest that *T. rubrum* possesses alternative metabolic pathways for the terminal oxidation of various substrates, one pathway is cyanide sensitive, the other is not.

References

Conant, N. F., Smith, D. T., Baker, R. D., Callaway, J. L. & Martin, D. D. (1954) Manual of clinical mycology, p. 336, 2nd ed., W. B. Saunders Co., Philadelphia, Pa.

Foster, J. W. (1949) Chemical activities of fungi, pp. 178, 555. Academic Press, Inc., New York, N.Y.

Frieden, E. & Maggiolo, I. W. (1957) Activation and other properties of ascorbic acid oxidase; Biochem. Biophys. Acta, **24,** 42—57.

Golden, M. J. & Oster, K. A. (1950) Studies on alcohol-soluble fungistatic and fungicidal compounds. V. Evaluation of fungistatic and fungicidal properties of antifungal ointments *in vitro*; J. A. Ph. A. (Sci Ed.), **39,** 47—50.

Hastings, A. B., Teng, C. T., Nesbett, F. B. & Sinex, F. M. (1952) Studies on carbohydrate metabolism in rat liver slices. I. The effect of cations in the media; J. biol. Chem., **194,** 69—81.

Horecker, B. L., Stotz, E. & Hogness, T. R. (1939) The promoting effect of aluminum, chromium and the rare earths in the succinic dehydrogenase-cytochrome system; J. biol. Chem., **128,** 251—256.

Kern, M. & Racker, E. (1954) Activation of a DPNH oxidase by an oxidation product of ascorbic acid; Arch. Biochem. Biophys., **48,** 235—236.

Krebs, H. A. & Eggleston, L. V. (1945) Metabolism of acetoacetate in animal tissues; Biochem. J., **39,** 408—419.

Lyon, I. & Geyer, R. P. (1954) Fatty acid metabolism and hepatic lipogenesis; J. biol. Chem., **208,** 529—536.

Lyon, I., Geyer, R. P. & Marshall, L. D. (1955) Further studies in fatty acid metabolism and hepatic lipogenesis; J. biol. Chem., **217,** 757—764.

McCallan, S. E. A. & Wilcoxon, F. (1934) Fungicidal action and the periodic system of the elements; Contr. Boyce Thompson Inst., **6,** 479—500.

McCallan, S. E. A. & Wilcoxon, F. (1939) Theoretical principles underlying laboratory tests of fungicides; Contr. Boyce Thompson Inst., **10,** 329—338.

McCallan, S. E. A. & Wilcoxon, F. (1940) An analysis of factors causing variation in spore germination tests of fungicides. I. Methods of obtaining spores; Contr. Boyce Thompson Inst., **11,** 5—20.

Melton, F. M. (1951) The effect of various substances on the oxygen uptake of *Microsporum canis* grown in submerged culture; J. Invest. Derm., **17,** 27—35.

Nickerson, W. J. (1946) Inhibition of fungus respiration: a metabolic bioassay method; Science, **103,** 484—486.

Nickerson, W. J. & Chadwick, J. B. (1946) On the respiration of dermatophytes; Arch. Biochem., **10,** 81—100.

Novak, M. V. & Hall, H. (1939) A method for determining the efficiency of preoperative skin sterilization; Surgery, **5,** 560—566.

Oster, K. S. & Golden, M. J. (1947) Studies on alcohol-soluble fungistatic and fungicidal compounds. I. Evaluation of fungistatic laboratory test methods; J. A. Ph. A. (Sci. Ed.) **36,** 283—288.

CONSIDÉRATIONS À PROPOS DES CARACTÈRES MYCOLOGIQUES ET PATHOGÉNIQUES DU KERATINOMYCES AJELLOI - VANBREUSEGHEM 1952, SAPROPHYTE DU SOL.

par

R. Evolceanu & I. Alteraş

(*Travail effectué dans la Section Dermatologique de l'Institut de Thérapeutique de l'Académie de la République Populaire Roumaine. Directeur de l'Institut: Prof.* N. Lupu, *membre de l'Académie Roumaine. Chef de la Section Dermatologique: Prof.* Stef. G. Nicolau, *membre de l'Académie Roumaine*).

(2.III.1959)

L'origine saprophytique des champignons parasites, les dermatophytes y compris, se confirme chaque jour davantage. Ce sont justement les dermatophytes dont cette origine paraît être le mieux démontrée. En effet, ces champignons, qui ne peuvent pas se développer que sur des tissus morts ou à vitalité amoindrie (comme la couche cornée de la peau et les phanères) sont plutôt des saprophytes en voie d'adaptation à la vie parasitaire.

Au milieu de la nature, les champignons se développent au dépens des restes organiques, qu'ils trouvent surtout dans le sol et l'on sait que le sol constitue non seulement un milieu favorable pour ces organismes mais, d'après les expériences de Vanbreuseghem & Van Brussel (1952), il se montre capable de régénérer des souches vieilles ou pléomorphisées. D'après Vanbreuseghem (19), les dermatophytes effectueraient une partie de leur cycle dans le sol comme saprophytes. En effet, déjà en 1940, Szathmary annonce avoir trouvé, dans la vase de certaines rivières, un champignon inoculable, voisin au *Ctenomyces interdigitalis*. De même, le *Ctenomyces mentagrophytes* a été trouvé dans le sol, en 1955, par Lurie & Borok. Mais, ce sont là des faits isolés. Il y a un autre dermatophyte, à savoir le *Microsporum gypseum* qui, comme saprophyte du sol, paraît être beaucoup plus répandu. Bien entendu, cette assertion est vraie seulement si les procédés actuels, utilisés en vue d'isoler ces champignons du sol, peuvent être acceptés comme universellement valables [1]). Ce *Microsporum gypseum* (*Sabourau-*

---

[1]) On peut se demander si, dans les couches superficielles du sol, il n'y auraient pas d'autres dermatophytes, tout aussi répandus mais qui se développeraient moins bien sur la kératine des cheveux et dont la mise en évidence par le procédé de Vanbreuseghem (voir plus loin) aurait moins de chances à réussir.

dites gypseus) on l'a trouvé, dans les couches superficielles du sol, un peu partout dans le monde. En 1953, AJELLO a isolé 37 souches de ce champignon, des terres riches en résidus organiques de Georgie et Tennesee (États Unis d'Amérique). Depuis lors, ce champignon a été isolé en Australie, au Cuba et en Europe. Récemment, EVOLCEANU & ALTERAŞ (1958) l'ont isolé en Roumanie, tant à Bucarest (en 9 endroits différents) qu'en province. Dans la plaine ou à la montagne (dans le sol des jardins et des forêts), partout où ils l'ont cherché, ils ont fini par le trouver.

En 1952, VANBREUSEGHEM a isolé, des couches superficielles du sol, en Belgique, un champignon non encore décrit et dont les caractères morphologiques et antigéniques étaient ceux des dermatophytes. Ce champignon, qu'il nomme *Keratinomyces ajelloi*, a été retrouvé dans les terres riches en résidus organiques, par G. DANIELS (1954) en Angleterre (1953) et par DURIE & FREY (1956) en Australie (1955). D'après VANBREUSEGHEM, ce champignon serait très répandu dans ces terres. VANBREUSEGHEM, GHISLAIN et WILLIAMS (1952) l'ont récemment isolé d'une lésion inflammatoire, banale de la peau. D'après ces auteurs, le *Keratinomyces* était ici un simple contaminant accidentel, ne jouant aucun rôle dans l'étiologie de cette inflammation.

Utilisant le procédé de VANBREUSEGHEM,¹) nous avons réussi à isoler le *Keratinomyces* des couches superficielles du sol. Les échantillons de terre, où nous l'avons découvert, ont été prélevés à Bucarest en deux endroits éloignés l'un de l'autre (la cour de l'hôpital ,,Colentina'' et le jardin botanique), situés sous des arbres et, par conséquent, riches en résidus organiques. Récemment nous l'avons trouvé dans un pot à fleurs. En outre, nous l'avons isolé d'une dyshidrose plantaire. Mais ici, comme chez le malade cité par VANBREUSEGHEM et col., et mentionné plus haut, le *Keratinomyces* ne jouait probablement aucun rôle dans l'étiologie de la dysidrose, car on a réussi à isoler, des vésicules plantaires, le *Ctenomyces interdigitalis*.

Des quatre souches de *Keratinomyces*, dont il a été question plus haut, nous avons étudié, du point de vue morphologique et pathogénique, les deux premières seulement.

CULTURES

Aspect macroscopique. Sur le milieu d'épreuve de Sabouraud, les colonies, à développement rapide, atteignant en 3—4 semaines 7—8 cm de diamètre, à surface uniforme, d'apparence finement plâtreuse et de coloration ocre, produisaient un pigment rouge-acajou diffusible (fig. 1). Ce pigment était visible d'abord à la partie profonde de la culture mais, assez rapidement, il diffusait dans le milieu. L'une des souches (celle isolée du jardin bota-

---

¹) Des cheveux sont mis en contact avec de la terre humide jusqu'à leur invasion par des moisissures. Contrôle microscopique des cheveux envahis et ensemencement des portions qui contiennent des macro- ou des microconidies.

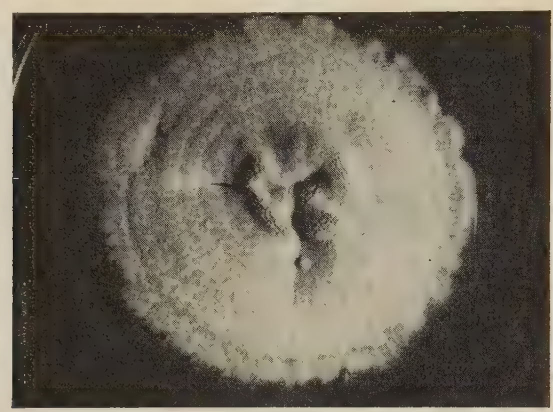


Fig. 1. *Keratinomyces ajelloi.* Culture de 3 semaines sur agar glucosé de Sabouraud, en matras d'Erlenmeyer.

nique) en était plus riche. Les milieux liquides se coloraient plus fortement et plus rapidement que les milieux solides.

Aspect microscopique. Cet aspect a été étudié sur des cultures en goutte pendante (chambre humide de Van Tieghem) sur milieu de Sabouraud (gélosé). Le mycélium, assez mince (3μ de largeur), présentait parfois la disposition dite en raquette. Le fait caractéristique pour la morphologie microscopique du champignon nous a paru être le grand nombre des macroconidies (fig. 2, et 3). L'abondance en ces organes qui devenaient visibles dès le 3-ème jour, était des plus frappantes. C'étaient des macroconidies à double contour, généralement de forme allongée, de couleur jaunâtre, à surface parfaitement lisse et divisées en logettes assez nombreuses (7—12). Leurs extrémités libres étaient amincies mais par places, elles étaient légèrement arrondies ou même en massue, comme chez l'*Epidermophyton floccosum.* Dans les cultures vieilles de 6 à 7 semaines, on pouvait parfois observer la séparation de la dernière logette, qui revêtait la forme d'une spore arrondie (fig. 2).

En un mot, ces macroconidies se différenciaient de celles propres au *Microsporum gypseum* par leur forme plus allongée, plus arrondie à leur extrémité libre, par leur parois plus lisses, par leur transparence plus grande et par le grand nombre des logettes. Leur disposition était en grappe simple et elles s'inséraient sur leur sporophore à l'aide d'un court stipe.

En ce qui concerne les aleuries, nous n'avons pas réussi à les mettre en évidence, jusqu' à présent, avec certitude. Les rares petites spores que nous avons observé dans les jeunes cultures et qui auraient pu être interprétées comme des aleuries, cessaient d'être visibles au bout de quelques jours.

Les souches isolées par nous et cultivées sur des cheveux ou de la laine, ont montré un pouvoir kératinolytique comparable à celui du *Microsporum gypseum* et du *Microsporum lanosum.* Comme on l'a vu plus haut, ces souches différaient très peu de celles qui ont

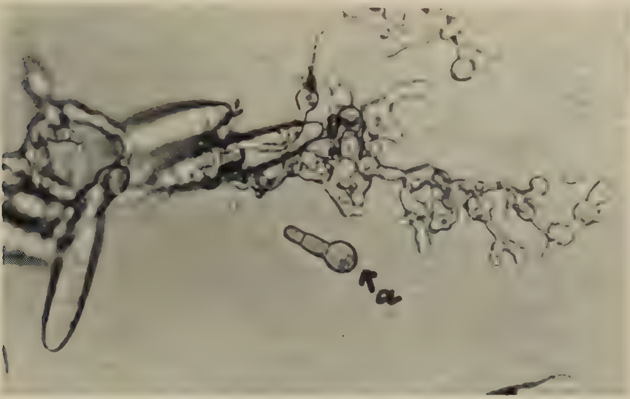


Fig. 2. *Keratinomyces ajelloi*. Macroconidies et cellules diformes. (culture en goutte pendante sur milieu de Sabouraud).

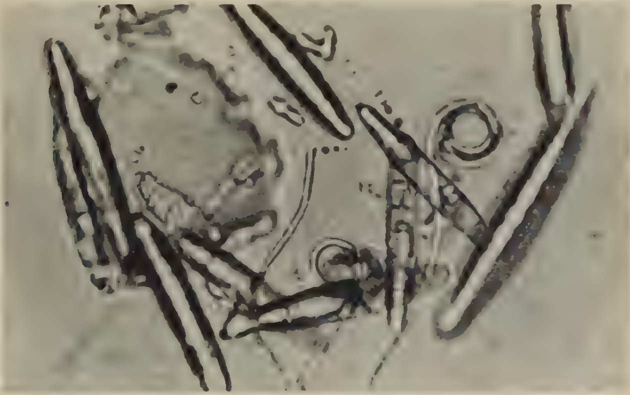


Fig. 3. *Keratinomyces ajelloi*. Macroconidies et vrilles serrées (culture de 6 semaines, en goutte pendante).

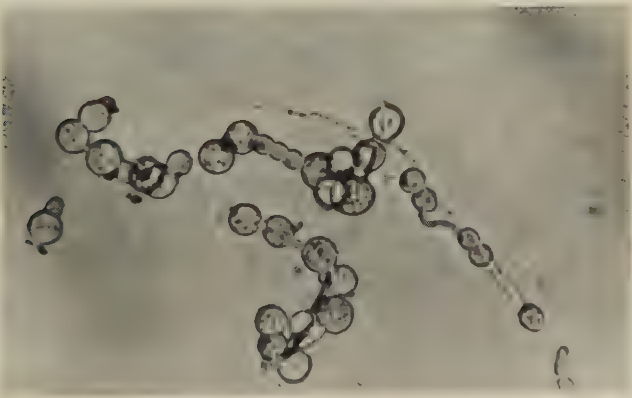


Fig. 4. *Keratinomyces ajelloi*. Chlamydospores disposées en chaînes (goutte pendante, culture de 20 jours).

servi à VANBREUSEGHEM comme modèle pour sa première description de ce champignon, les macroconidies de nos souches ayant leurs extrémités libres moins amincies. En outre, une de nos souches montrait de rares vrilles serrées qui apparaissaient sur les milieux usuels (fig. 3) et des chlamydospores en chaînes (fig. 4).

ESSAIS D'INOCULATIONS EXPÉRIMENTALES

Nous les avons fait à la souris blanche, au cobaye et à l'homme. Seulement l'une de nos deux souches s'est montrée pathogène, à savoir celle que nous avons isolé du jardin botanique. C'était la souche dont les cultures ont produit plus de pigment mais, en revanche, à morphologie un peu moins riche, puisque nous n'y avons pas trouvé ni vrilles, ni chlamydospores. Avec cette souche, les inoculations ont réussi, bien que difficilement, tant aux animaux, qu'à l'homme. Il faut avouer qu'il nous a fallu faire de nombreux essais et sur des assez nombreux individus pour réussir une seule fois chez l'homme, de même que chez la souris blanche et le cobaye. Les inoculations ont été effectuées, par le procédé classique, en frottant la peau avec du papier de verre, sur lequel nous avons déposé des cultures finement triturées. Les animaux ont été inoculés sur le dos, après dépilation mécanique; l'homme, sur la face antérieure de l'avant-bras.

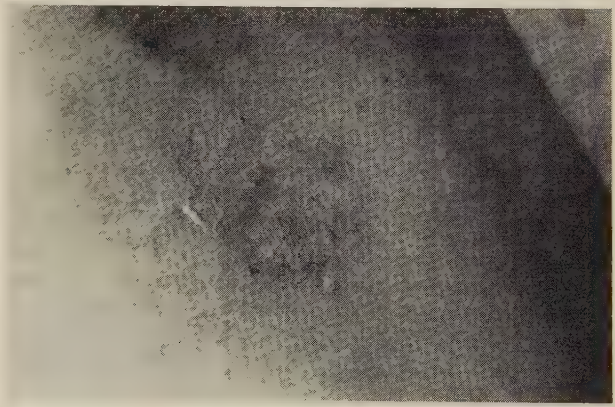


Fig. 5. Inoculation expérimentale du *Keratinomyces ajelloi* à l' homme (avant-bras). Lésion du type herpès circiné.

Chez l'homme, nous avons constaté, dès le 5-ème jour, l'apparition d'une tâche rouge, légèrement infiltrée, à la surface de laquelle ont apparu, les jours suivants, des vésiculo-pustulettes ayant les dimensions d'une tête d'épingle, l'ensemble de la lésion revêtant l'aspect d'un herpès circiné (fig. 5). Les pustulettes contenaient de nombreux filaments mycéliens, assez gros. Ces lésions ont disparu après 5 à 6 jours, sans aucun traitement.

Chez le cobaye et chez la souris blanche, la tâche rouge est apparue au bout de 7 à 8 jours après l'inoculation. Elle était couverte de squames fines qui contenaient de gros filaments mycéliens. La disparition de la lésion s'est produite au bout de 7 à 8 jours. Les poils n'ont pas été envahis.

Il faut souligner le fait que chez l'homme, tout aussi bien que chez l'animal, les filaments mycéliens, qui étaient présents en grand nombre dans les lésions expérimentales, avaient un diamètre beaucoup plus gros que celui des filaments qui se trouvent dans les cultures (fig. 6). Ils étaient bien constitués et pas du tout altérés. ajoutons encore que nous n'avons pas retrouvé des macroconidies ni dans les vésicules, ni dans les squames. Nouscroyons que ces constatations suffisent à éliminer la supposition suivant laquelle les filaments en question n'auraient représenté que de simples restes de la culture inoculée.

L'ensemencement des vésicules et squames, récoltées des lésions expérimentales, ont reproduit exactement la souche qui a été inoculée.

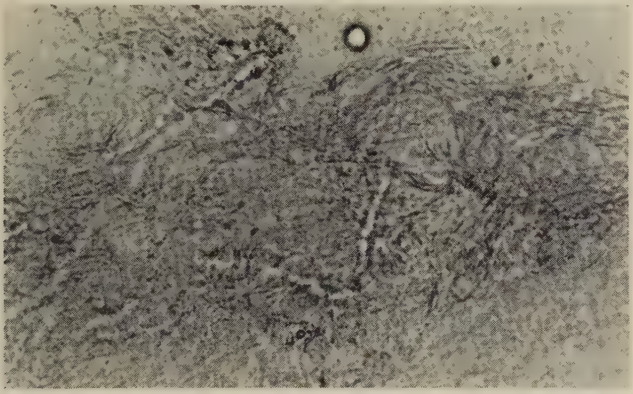


Fig. 6. Lésion expérimentale chez l'homme: rares filaments mycéliens (parois des vésicules).

COMPORTEMENT DU KERATINOMYCES AJELLOI COMME ANTIGÈNE

Nous avons préparé un extrait des cultures de nos souches développées sur milieu liquide de Sabouraud et vieilles de 5 à 6 semaines. On a utilisé seulement la masse mycélienne qui, après avoir été bien lavée à l'eau courante, a été deshydratée en la maintenant 24 heures en acétone, puis séchée et finement triturée. La poudre qui en résultait était délayée dans l'eau distilée, en proportion de 1/9, maintenue à 80° C pendant une heure et centrifugée. Le surnatant était soumis à la dialyse à l'eau courante, pendant 2 jours, puis à l'eau distilée et enfin séchée.¹)

---

¹) Cette méthode a été créée par E. SORU en vue d'extraire les toxines bactériennes. Dans notre service on l'emploie, avec succès, pour préparer la trichophytine en poudre.

Notre extrait, dilué a 1/500 et administré par voie intradermique à un malade atteint de mycose inflammatoire de la peau, a provoqué une réaction positive, tandis que chez les individus témoins cette même réaction a été négative. Par conséquent, nos souches se sont comporté, du point de vue antigénique, comme une vraie trichophytine.

Discussions

La place qui revient au keratinomyces ajelloi dans la systematique des dermatophytes.

Les caractères morphologiques et la manière de ce comporter comme antigène nous obligent à placer, avec Vanbreuseghem, cette espèce parmi les dermatophytes communs, à savoir parmi les „trichophytonées" (ainsi nommées par C. W. Dodge). Il n'y a aucun doute que ce dermatophyte soit plus primitif que tous les dermatophytes isolés jusqu'à présent, puisqu'il ne figure pas parmi les agents des mycoses connues, autrement dit il ne s'est pas encore, véritablement, adapté à la vie parasitaire. A côté du dermatophyte désigné sous le nom de *Microsporum gypseum*, le *Keratinomyces ajelloi* est essentiellement un saprophyte du sol. Si l'on tient compte de l'existence, chez celui-ci, des vrilles serrées, ainsi que de l'absence des aleuries, compensée par le grand nombre des macroconidies, dont la forme paraît être intermédiaire [1]) entre celle des macroconidies du genre *Sabouraudites* et celle du genre *Epidermophyton* — Ota et Langeron 1923, on serait tenté d'admettre que le *Keratinomyces ajelloi* est un *Epidermophyton* primitif. D'ailleurs, la seule espèce connue du genre *Epidermophyton* — Ota et Langeron, à savoir l'*Epidermophyton floccosum* — Harz 1870 est, elle aussi, très difficilement inoculable par voie expérimentale. D'autre part, nous n'avons pas trouvé le *Keratinomyces* dans les poils des individus (hommes et animaux) chez lesquels l'inoculation expérimentale a donné un résultat positif. Selon nous, en vue de simplifier la systématique, il sérait avantageux de considérer ce champignon comme un *Epidermophyton* et de le désigner sous le nom d'*Epidermophyton ajelloi* ou, mieux encore, sous le nom d'*Epidermophyton terrigenum*, cette dernière dénomination indiquant, en même temps, son origine tellurique.

Si, d'après Vanbreuseghem, le *Keratinomyces ajelloi* est très répandu dans le sol, il paraît qu'au moins chez nous, en Roumanie, il doit céder la place, de ce point de vue, au *Microsporum gypseum*. En effet, le premier nous ne l'avons isolé du sol que 3 fois, tandis que le second nous l'avons trouvé partout. Toutes les portions de terrain, couvertes de végétation, à Bucarest et en province, situées à des altitudes les plus diverses (jusqu'à 1500), nous ont révélé la présence de ce dernier dermatophyte.

---

[1]) L'extrémité libre de macrocomidies de nos deux souches étaient moins amincies que celles propres au genre *Sabouraudites* (*Microsporum*). De plus, dans les cultures vieilles, nous avons parfois observé la séparation des logettes des macroconidies (surtout de la dernière) et leur transformation en spores arrondies. D'après ce que nous avons observé chez le *Sabouraudites*, les logettes de macroconidies germent sur place sans se détacher, tandis que chez l'*Epidermophyton*, elles se détachent facilement.

D'autre part, la pathogénité du *Keratinomyces ajelloi* est, comme on l'a vu plus haut, prouvée, et l'on peut en conclure qu'il est un dermatophyte authentique, bien qu'il soit, à l'heure actuelle, le moins adapté à la vie parasitaire. L'ubiquité du *Keratinomyces ajelloi* et du *Microsporum gypseum,* dans les couches superficielles du sol, étant un fait bien établi et, par cela même, leur nature saprophytique on est forcé d'admettre que ce sont les types les plus primitifs des dermatophytes, plus primitifs probablement que le *Ctenomyces mentagrophytes.* Cela veut dire qu'à l'avenir tout essai nouveau de classification rationelle (phylogénétique) des dermatophytes doit tenir compte de ce fait et chercher d'établir la filiation de ces champignons à partir du *Keratinomyces* et du *Microsporum gypseum.*

CONCLUSIONS

1. Les auteurs ont isolé, à Bucarest, des couches superficielles du sol, où il vivait comme saprophyte, un dermatophyte qui, d'après ses caractères morphologiques, doit être considéré identique au *Keratinomyces ajelloi* — VANBREUSEGHEM 1952. C'est la première fois qu'on a isolé cette espèce en Roumanie.
2. Contrairement aux résultats obtenus ailleurs, concernant la pathogénité de ce champignon, considéré jusqu'à présent non pathogène pour l'homme et l'animal, l'une des souches isolées à Bucarest s'est montrée capable de provoquer, tant à l'homme, qu'au cobaye et à la souris blanche, une mycose expérimentale réelle, bien que très passagère.
3. Les auteurs considèrent qu'il n'est pas absolument nécessaire de créer pour cette espèce un genre nouveau. Étant donné, d'une part, sa morphologie microscopique et, d'autre part, sa capacité de déterminer des lésions épidermiques sans envahir les poils, les auteurs proposent de ranger cette espèce dans le genre *Epidermophyton* — OTA et LANGERON 1923, et de la désigner sous le nom d'*Epidermophyton terrigenum.*

RÉSUMÉ

Les auteurs ont signalé, pour la première fois en Roumanie (à Bucarest), la présence dans les couches superficielles du sol du *Keratinomyces ajelloi* — VANBREUSEGHEM 1952. La morphologie microscopique de l'une des deux souches, étudiées de ce point de vue, était un peu plus riche que celle de la souche isolée, pour la première fois par VANBREUSEGHEM car, outre les macroconidies, on y trouvait des vrilles serrées et des chlamydospores. D'autre part, chez les deux souches, les extrémités libres des macroconidies n'étaient pas constament amincies et, par places, plutôt arrondies. L'une des souches s'est montrée pathogène pour l'homme, le cobaye

et la souris blanche, chez lesquels son inoculation experimentale a provoqué une mycose superficielle authentique, bien que passagère. Ce caractère n'a pas été signalé jusqu'à présent. Vue sa morphologie microscopique, sa pathogénité et son origine tellurique, les auteurs proposent, pour cette espèce, le nom d'*Epidermophyton terrigenum*.

Summary

The authors report, for the first time in Rumania, the presence in the soil of *Keratinomyces ajelloi* — Vanbreuseghem 1952, isolated by them on 3 different places at Bucharest. One of the isolated strains showed a little more abundant microscopical morphology than the strain first isolated by Vanbreuseghem, for it had also coils and chlamydospores.

Although this new dermatophyte has been considered, up to now, nonpathogenic for man and animal, the authors were able to obtain positive results in white mice, guinea pig and in man, inoculating repeatedly one of the isolated strains. The experimental lesion it produced, of herpes circinatus type, disappeared in a few days.

As the authors conclude, it is not necessary to create a new genus for this dermatophyte. Considering its microscopical aspect, its pathogenicity and its soil origin, they propose to classify *Keratinomyces ajelloi* in the genus *Epidermophyton* — Ota et Langeron 1923 —, giving it the name of "*Epidermophyton terrigenum*".

Bibliographie

Arievitch, A. M. & V. N. Pentkovskaïa. Voprosî dermatologuii i venerologuii, **X**, 100—101, Medguiz-Moscou.

Daniels, G. Isolation of Keratinomyces ajelloi from the soil Great Britain. Nature 1954, **176,** 4422, 224—226.

Durie E. B. & B. M. Frey. Isolation of Microsporum gypseum and of Keratinomyces ajelloi from australian soil. Nature (London) **17** 1956, p. 4484

Evolceanu R. & I. Alteras. Le Microsporum gypseum. Quelques observations cliniques et mycologiques concernant ce dermatophyte. Mycopathol. Mycol. appl. **X,** 2, 71.

Evolceanu R., A. Avram, I. Alteras, T. Roxin. Dermatomicoze. Editura Medicală, Bucureşti 1956, 62—63.

Kachkine P. N., Dermatomikozî Medghiz Leningrad 1954, 154—155.

Lurie H. I. & R. Borok. Mycologia (New-York) **47,** 50, 6, 1955.

Szathmary S., Trichophyton fluviale. Arch. f. Dermatol. Syphilis **181,** 2, 1940, p. 192.

Vanbreuseghem R., Intérêt théorique et pratique d'un nouveau dermatophyte isolé du sol: Keratinomyces ajelloi, gen. nov. sp. nov. Bull. de la Classe des Sciences 5-ème série, **XXXVIII** 1952. 1068—1077.

Vanbreuseghem R., Les champignons kératinophyles et kératinolytiques. Atti VI Congr. Int. Microbiol. Vol. **V** pag. 236, Sezione XIV Miceti Roma 6—12 sett.95.

Vanbreuseghem R., Arch. Belges Dermatol. Syphiligraphie. c. **XIII,** 4, décembre.

Vanbreuseghem R., E. Ghislain & W. Williams. Signification de l'isolement d'une souche de keratinomyces ajelloi Vanbreuseghem 1952, à partir de l'homme Arch. Belges Dermatol. Syphiligraphie, **XII,** 2, 1952, 130—134

Vanbreuseghem R. & M. Van Brussel. Réversibilité du pleomorphisme des dermatophytes. C. R. Soc. Biol. 1952, 146.

OBSERVATIONS ON PSYCHONEUROPHYSIOLOGICALLY SIGNIFICANT MUSHROOMS

SAM I. STEIN, GERHARD L. CLOSS AND NORMAN W. GABEL

I. CLINICAL DETAILS PERTAINING TO THE INGESTION OF PANAEOLUS VENENOSUS AND PSILOCYBE CAERULESCENS MUSHROOMS

SAM I. STEIN, M. D., Ph. D.[1])

(14.IV.1959)

Introduction

The exploration of the effects of psychoneurophysiologically significant mushrooms or the chemicals they contain is continuing and ostensibly expanding (1) (2) (3). In some instances the most dramatic and subjectively discernible effect was in the psychic modality of the nervous system. Probably for this reason, the term "hallucinogenic" was applied to categorize the mushroom effects with those produced by other naturally occurring plant substances (mescaline, LSD 25, Yohimbine) under experimental investigation with the advent of the psychopharmacological era which started about 1952. The term "hallucinogenic" is unfortunate since (a) it suggests the probability of having material capable of causing effects identical to those obtaining in dementia praecox which can thereby be studied at will; and (b) it misleads by placing an emphasis on the effects in the psychic modality and thereby deters observations of the effects in other areas of the nervous system or in other systems. The need for having qualified screening measurements for psychopharmacological effects is of paramount importance (4). In view of this requirement, this case study reports the clinical details as related, measured, and observed on a non-patient volunteer, Dr. GERHARD L. CLOSS, Assistant Professor of Chemistry, The University of Chicago, who was interested in personally experiencing the effects of our mushrooms whose significant chemicals he is seeking to isolate and identify in his laboratory.

Experimental Studies

Dr. CLOSS, the subject, is 30 years of age. The following was obtained in sequence.

August 17, 1958: First Experiment

---

[1]) Coordinator of Research and Clinical Investigator, The Bertram and Roberta Stein Neuropsychiatric Research Program, Inc.

9 : 50 A.M.

Room temperature:	72° F
Room humidity:	50 %
Pulse:	80
Blood pressure:	128/60—70
Weight:	155
Pupils:	Moderately dilated; react quickly to light and accommodation

9 : 55 A.M.

Subject was requested to:	Draw A Person (DAP)
Subject was requested to:	Draw A Person of the Opposite Sex (DAPOS)

10 : 10 A.M.

Subject took 0.5 Gm. *Panaeolus venenosus* [1])

10 : 20 A.M.

Pulse:	83
Blood pressure:	112/70
Pupils:	Slightly dilated
Subject reports:	Slight dizziness

10 : 35 A.M.

Pulse:	68
Blood pressure:	120/72
Subject reports:	Slight inebriation. Turning causes dizziness. See subject's notes.

10 : 50 A.M.

Pulse:	60
Blood pressure:	118/76
Subject reports:	"The effect is decreasing. I feel normal".

11 : 20 A.M.

Pulse:	64
Blood pressure:	105/70

August 22, 1958: Second Experiment

9 : 20 A.M.

Check normal polygraphic effects. (see tracing)

9 : 30 A.M.

Pulse:	70
Blood pressure:	115/65
Weight:	155
Oral temperature:	98.3° F
Room temperature:	72° F
Room humidity:	52 %
Pupils:	Approximate size noted

9 : 36 A.M.

Subject took 1.0 Gm. of *Panaeolus venenosus* [2])

9 : 46 A.M.

Pulse:	73
Blood pressure:	110/56
Pupils:	Seemingly unchanged

---

[1]) Oven-dried. Identified by Drs. ROLF SINGER, University of Argentina, Tucuman, and A. H. SMITH, University of Michigan; grown by Dr. L. KNEEBONE, Pennsylvania State University.

[2]) Same as material used one August 17, 1958.

9 : 55 A.M.
Pulse: 74
Blood pressure: 110/72
Pupils: Slightly more dilated
Subject reports: No effects and inquires if he had been given bread crumbs.

10 : 05 A.M.
Subject reports voluntarily: "I feel different". Slight dizziness. Tingling of fingers. Feels warm. Sweating.
Pupils: Distincly larger
Pulse: 60
Blood pressure: 120/75

10 : 12 A.M.
Subject volunteers: "It's a type of intoxication, hard to describe". He uses his hands to maintain equilibrium while walking. "It's more pronounced than last time. The intoxication is not unpleasant at all." Lights a cigarette.

10 : 15 A.M.
Pulse: 66
Blood pressure: 108/74
Walking: Equilibrium disturbed
Subject reports: "Bitter feeling at tip of tongue".

10 : 20 — 10 : 30 A.M. (polygraphic tracings)
Subject reports: The paresthesias are still present. "The total effect is more pronounced than last time. Not in the mood to solve any complicated chemical problems. Just feel like relaxing". Attitude is one of relative indifference or diminished motivation. The effect seems to be at its height at this time.

10 : 32 A.M.
Subject is asked to: Draw A Person which he attempts but comments: "I have difficulty concentrating on one spot with my eyes. It seems as if I am not able to draw at all today". Stops drawing. His hands feel warm and completely wet from sweat. He lolls back. Claims to sense anxiety and states: "I can't figure out (name) the problems going on in the lab now".

10 : 37 A.M.
Pulse: 58—60
Blood pressure: 108/70
Oral temperature: 97.9—98° F
Pupils: Still slightly dilated
Walking: Unsteady: "It is not like from weakness, but disturbed equilibrium".

10 : 48 A.M.
Subject was requested to: DAPOS. His movements are active now, and he is showing his more usual interest in responding.

10 : 55 A.M.
Subject eats some fresh seedless grapes and a plum.
Subject reports: No gastro-intestinal effects but states that paresthesias are still present in his fingers.

11 : 00 A.M.
Subject is smoking. Now is motivated and capable of discussing the work of his laboratory.
Pulse: 54
Blood pressure: 116/76
Hands: Warm, but sweating less.
Pupils: Still slightly dilated but react briskly to light now.
Subject reports: "The intrinsic sensation is much less and equilibrium is better".

11 : 30 A.P.
Pulse: 54
Blood pressure: 110/74

12 : 00 Noon
Pulse: 56
Blood pressure: 110/74

12 : 45 P.M. (lunch)
Pupils: Dilated. Give distress in outdoor light.

1 : 30 P.M.
Pulse: 62
Blood pressure: 110/64
Pupils: Nearly original size.
Subject drinks lemonade

2 : 00 P.M.
Pulse: 72
Blood pressure: 114/70
Pupils: Nearly normal. Out-door light gives no distress.

2 : 45 P.M.
Pulse: 66
Blood pressure: 118/64
Oral temperature: 98.1° F
Pupils: Normal

August 29, 1958: Third Experiment

Room temperature: 71° F
Room humidity: 58 %

9 : 35 A.M.
Pulse: 70—71
Blood pressure: 112/60
Weight: 154 plus
Oral temperature: 98.3° F
Pupils: Observed for size and tested for light and accommodation reflex.

9 : 45 A.M.
Subject ingested 1.0 Gm. oven-dried, granulated *Psilocybe caerulescens* (i.e. prepared and identified in the same way as the *Panaeolus venenosus* used in the previous experiments).

9 : 50 A. M.(yawning)
Pulse: 77
Blood pressure: 110/56

10 : 05 A.M.
Pulse: 72
Blood pressure: 108/64
Subjective effects: None reported

10 : 15 A.M.
Pulse: 64
Blood pressure: 104/60
Pupils: Slightly dilated
Skin effects: None
Equilibrium: Questionably affected
Subjective effects: "Unpleasant sensation in the tube from mouth to stomach". Subject was given some water.

10 : 20—10 : 25 A.M.
Subject smoking a cigarette.

10 : 27 A.M.
Pulse: 64
Blood pressure: 102/74
Subjective effects: Subject is yawning and complains of some dizziness but mainly fatigue.

10 : 35 A.M.
Subject comments: „The equilibrium system is not as affected. There is some sense of inebriation but more like the 0.5 Gm. portion of *Panaeolus venenosus*. The main feeling-tone at this time is tiredness". There is yawning and a drooping of eyelids. This yawning and somnolence with *Psilocybe* was observed by the author upon himself (5) and another subject (3).

10 : 39 A.M.
Pulse: 65
Blood pressure: 108/70
Subjective effects: "The sensations are different from *Panaeolus*. This makes me feel terribly tired. This is like the normal state of being tired. With *Panaeolus*, it was a relaxed feeling".

10 : 50 A.M.
Pulse: 64
Blood pressure: 105/70
Subjective effects: "I have had some unpleasant effects in the stomach, not serious, but noticeable. The main sensation is that I wish to take a nap. That's very pronounced". At this moment a stranger entered the office-suite, and Dr. C. stood up to observe a demonstration. A manner of intoxication seemed to prevail.

11 : 05 A.M.
Pulse: 65
Blood pressure: 108/68
Subjective effects: "I have an intoxicated feeling. The equilibrium isn't much affected. I feel strange and very drunk now". Facial expression appears drawn or anxious. Dr. C. does not report any visual hallucinations or any haloeffects as he looks at the light.

11 : 10 A.M.
Subject was attached to the polygraphic apparatus, since the effects appeared to have reached a significant stage or one of strong intoxication. The polygraph had run only a few minutes when:
Subject reported: "Something strange is happening. I feel real drunk, and I have strong anxieties".

Hands: Cool but sweating

Subject was removed from the polygraph.

Pulse: 72

Blood pressure: 105/64

Oral temperature: 98° F

Because of subjective sensations described above, he was given fresh fruit (grapes and plums).

11 : 40 A.M. Reading taken immediately after Dr. C. ate the fresh fruit.

Pulse: 48

Blood pressure: 98/55

Pupils: Dilated

11 : 50 A.M.

Subjective effects: "I feel cold, even my hands and nose". There is evidence of some shivering.

Room temperature: Still 72° F

Room humidity: 52 %

11 : 55 A.M.

Pulse: 56

Blood pressure: 105/68

Oral temperature: 98.3° F

Subjective effects: Intoxication symptoms subsided rapidly after this.

12 : 25 P.M.

Pulse: 58

Blood pressure: 104/62

Subject ate lunch immediately after reading.

1 : 40 P.M.

Pulse: 74

Blood pressure: 112/64

Pupils: Still slightly dilated but reacted quickly to light stimulus.

Summary

There were noticeable similarities and differences in the effects of *Panaeolus venenosus* and *Psilocybe caerulescens* which appeared to have no correlation with the quantity of raw mushroom ingested.

In the three experiments reported here, the subjective and objective effects developed within about the same time interval after ingestion; namely, 25 to 30 minutes, with one exception. Yawning, which was observed here and elsewhere as occurring only with the *Psilocybe* material, usually within 5 to 10 minutes, proceeds until other symptoms and signs present themselves.

Reported symptoms or subjective effects which occurred only or mainly with *Panaeolus* were (a) a mild or moderate relaxing (tranquil) type of inebriation, (b) disturbed equilibrium, (c) either diminished motivation or blocking in the psychic (thought) process, and (d) paresthesias.

Features which obtained only or mainly with *Psilocybe* were (a) the aforementioned yawning in the induction period (b) "burning" sensation in the esophageal and stomach areas, (c) a feeling of extreme tiredness, and (d) a feeling-tone of anxiety bordering on collapse associated with a sense of strong intoxication.

The factors occurring in common were (a) the extreme reduction in the pulse rate, slightly more with *Psilocybe*, (b) the steadiness of the systolic and diastolic components of the blood pressure except for a very period of drop in both diastole and systole with *Psilocybe* during the period of strong intoxication, (c) dilation of the pupils, (d) a drop in body temperature, seemingly more marked with *Panaeolus*, but producing a much more subjective effect (feeling of cold) during the period of *Psilocybe* intoxication, and (e) sweating.

With *Panaeolus* there was a general drop in the total personality integration, but the internal sensation was a pleasant and relaxed feeling; whereas, with *Psilocybe* there was actually disorganization associated with the feeling of panic and disagreeable intoxication.

A most significant feature is that Dr. Closs at no time perceived or reported effects that could be categorized as hallucinatory.

Formulation

Obviously, it is unwise and perhaps unnecessary to try to correlate clinical observations where unknown chemicals or chemical complexes are ingested.

But the author has had considerable experience with psychoneuropharmacological (psychoneurotropic) material and offers the following tentative opinion. The relaxing subjective effects, the hypothermia, and diminished pulse found with *Panaeolus* resemble the effects attributed to serotonin. But the inebriation and the pupillary mydriasis cannot be explained as effects of serotonin. Some of the effects observed here (and elsewhere) (1) (3) with *Psilocybe* are probably due to the serotonin-related-psilocybin already isolated (6) from *Psilocybe mexicana* (Heim) which probably also obtains in *Psilocybe caerulescens*. (Refer to Part II by Dr. Closs) However, it seems unlikely that psilocybin will be found to have caused the signs of mydriasis and cerebral or cortical intoxication

Probably several chemical compounds will be found to be psychoneurophysiologically effective in each significant mushroom. In addition, undoubtedly, the relative differences in the biochemistry which obtain from individual to individual may account further for differences elicited, alleged or reported, including extrasensory perception and hallucinosis, in situations where so-called "sacred" (2) (7) mushroom material has been ingested.

II. STUDIES TOWARD THE ISOLATION OF THE ACTIVE CONSTITUENTS OF PANAEOLUS VENENOSUS

Gerhard L. Closs[1]) & Norman W. Gabel

The observed clinical effects described in Part I of this communication seemed to justify an attempt at the isolation and characterization of the compounds which are responsible for the remarkable action of *Panaeolus venenosus* upon the nervous system. The guiding principle in this work was the consideration that it would be highly desirable to obtain any of the compounds in their original form, unchanged from the state in which they occur in the mushroom. Since it seemed reasonable to expect that the active compounds might possess easily hydrolizable groups which could be of importance to their physiological activity, isolation and purification procedures had to be chosen to avoid any acid or base treatment.

---

[1]) Department of Chemistry. The University of Chicago, Chicago 37, Ill.

It should be pointed out that the experiments to be described are preliminary in nature and were designed to establish the best conditions for the isolation and purification of the compounds occurring in the mushroom. As a second stage, it is planned to work up larger quantities of the mushroom in order to obtain sizeable amounts of the compounds and test their activities in similar experiments as described in Part I. Unforeseen difficulties, however, prevented us from securing a large enough supply of *P. venenosus* to continue our investigations on a larger scale and made further characterizations of the isolated products impossible at the present time. The total amount of dried mushrooms which were available for our studies was less than 12 grams.

Paper Chromatography Studies on Extracts from Several Panaeolus and Psilocybe Species

At the outset it was necessary to establish whether the extractable compounds from *P. venenosus* were identical with those occurring in other *Panaeolus* species and particularly whether psilocybin (6) would be among them. Paper chromatography proved to be a valuable and reliable tool in these investigations.

The methanol extracts of the well-dried fruiting bodies were partially evaporated in vacuum, and fatty material was removed by extraction with hexane [1]). The methanolic solutions were then spotted on Whatman No 1 paper in suitable concentrations. As eluting solvent, isopropanol-water 9:1 was found most satisfactory. Spraying with a solution of p-dimethylamino-benzaldehyde (Ehrlich's reagent) followed by exposure to hydrogen chloride vapor, was used as detecting reagent. In several chromatograms Dragendorff's alkaloid reagent was used for detection.

Figure 1 shows the chromatograms of extracts from *P. venenosus, P. sphrinctrinus, Psil. mexicana, Psil. cubensis,* and *Psil. caerulescens.* All three *Psilocybe* species investigated show a strong spot at Rf 0.63 which produces a bluish-grey color with Ehrlich's reagent indicating the presence of psilocybin (6). A few very faint spots at lower Rf show the presence of minor constituents in much smaller concentrations.

---

[1]) Subsequent paper chromatograms of these extracts did not show any spots with the developing reagents used.

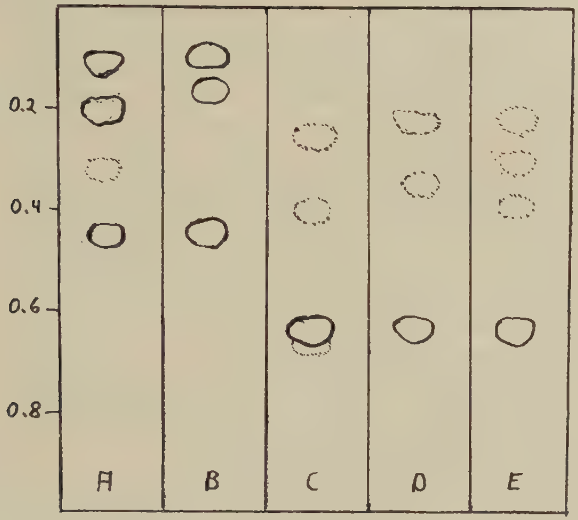


Fig. 1

Paper Chromatograms of A. *P. venenosus*, B. *P. sphrinctrinus*, C. *Psil. mexicana*, D. *Psil. cubensis*, E. *Psil. caerulescens*. Solvent: Isopropanol — water 9 : 1. Detecting reagent: Ehrlich's reagent.

The chromatograms of the *Panaeolus* species show clearly that psilocybin is absent. The chromatogram of *Panaeolus venenosus* has three strong spots at Rf 0.44, 0.20, and 0.11. The last two show up in blue color with Ehrlich's reagent, whereas the first appears as a bright yellow spot. The presence of at least one minor constituent is indicated by a faint spot at Rf 0.32. *Panaeolus sphrinctrinus* gives rise to a chromatogram with three intense spots at Rf 0.43 (yellow), 0.17 (red), and 0.09 (bluishgrey). It appears that the spots at Rf 0.4 in the chromatograms of both Panaeoli are produced by the same compound as judged by their identical Rf and color reactions with Ehrlich's reagent. However, the other major constituents in both *Panaeolus* species seem to differ from each other since they appear at different Rf values and produce different colors with the developing reagent. From these results it can be concluded that the extractable constituents of *Panaeolus venenosus* are totally different from the constituents of the *Psilocybe* species and partially different from the extracts obtained from *P. sphrinctrinus*.

Column Chromatogram of the Extract Obtained from Panaeolus Venenosus

Encouraged by the good resolution obtained on paper, it was decided to try the separation on a larger scale by chromatography on a cellulose column. The methanol extract from 11 grams of dried and finelypowdered mushrooms was evaporated under vacuum. Some of the high molecular weight material was removed from an aqueous solution of the residue by precipitation with ethanol. The resulting solution was evaporated under reduced pressure and the residue chromatographed on cellulose powder. As eluting solvent, isopropanol-water 9:1 was followed by 95 % ethanol. In good agreement with the paper chromatography studies, three major fractions were obtained. Paper chromatography of these fractions demonstrated their correspondence to the three major spots of the paper chromatogram of the total extract. A fourth fraction, corresponding to the minor spot on paper at Rf 0.32, could be recognized but was not further investigated because of the very small quantity of this material. Each of the major fractions was rechromatographed several times, and two of the three compounds were finally obtained crystalline.

"Compound I", first compound to be eluted from the column, could be shown to be identical with the substance producing the yellow spot on paper at Rf 0.44. It was obtained from ethanolethyl acetate in tiny needles and could be further purified by sublimation in vacuum. The compound melts at 136° C, shows no ultra violet absorption, and has the typical characteristics of a hydrohalide (insoluble in nonpolar solvents, infra red spectrum with strong absorption at 3300—3000 and 1600 cm-^{1} and ionic chlorine).

The next larger fraction to be eluted from the column could be shown to correspond to the blue spot on paper with Rf 0.20. This *"Compound II"* was obtained crystalline, after considerable losses, on purification by rechromatographing it several times on cellulose. The compound decomposes above 250° C, is soluble in water, insoluble in nonpolar solvents, and could be recrystallized from ethanol. The compound is not extractable either from acidic or basic solutions, suggesting acidic and basic functions in the molecule. Not enough material was available to obtain a quantitative elementary analysis. The ultra violet absorption spectrum (Figure 2) shows maxima at 220, 265, and 288 mu. The band positions and relative intensities agree well with the spectrum to be expected for a benzene hydroxylated indole chromophore. The blue color reaction with Ehrlich's reagent supports this conclusion. It should be noted that the spectrum is almost identical with the spectrum of psilocybin (6). This correlation indicates that Compound II possesses the same chromophore as psilocybin, an esterified 4-hydroxy indole system. This near relation to psilocybin seems to suggest that Compound II is the active principle of *P.*

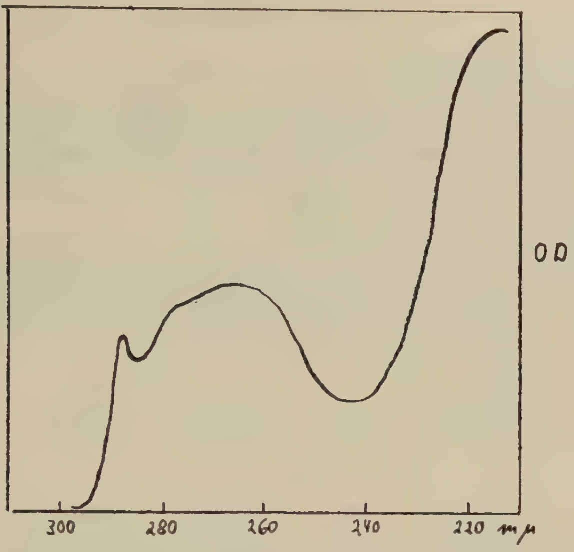


Fig. 2
Ultra Violet Absorption Spectrum of Compound II in Methanol.

venenosus although actual tests of its activity could not be carried out because of the small amount isolated. From its greater solubility in water, higher melting point, and smaller Rf value, it can be concluded that the compound is more polar than psilocybin. From the ultra violet spectrum of the total extract which is very similar to the spectrum of Compound II and shows the characteristic band at 288 mu very clearly, the total amount of Compound II present in the mushroom can be estimated to 0.1—0.3 %.

The last fraction to be eluted from the column did not yield a crystalline compound. Paper chromatography demonstrated that it corresponds to the blue spot at Rf 0.11. The ultra violet absorption spectrum of this material showed a maximum at 260 mu. The material was not further investigated.

Summary

The extracts of *P. venenosus*, *P. sphrinctrinus*, *Psil. mexicana*, *Psil. cubensis*, and *Psil. caerulescens* have been compared by paper chromatography. It was found that neither Panaeoli contain psilocybin, the main constituent of the three *Psilocybe* species. The extract from *P. venenosus* has been chromatographed, and two of the three major constituents were purified and obtained crystalline. One of these compounds could be shown to possess the same chromophore as psilocybin, a 4-oxygenated indole system, and seems most likely to be the active compound of the mushroom.

BIBLIOGRAPHY

1. HEIM, ROGER, Revue de Mycologie. Laboratoire de Cryptogamie, Museum National D'Histoire Naturelle, Paris. April 15, 1958.
2. PUHARICH, A. The Sacred Mushroom. Doubleday, New York, 1959.
3. STEIN, S. I. "Clinical Observations On the Effects of Panaeolus venenosus Versus Psilocybe caerulescens Mushrooms". Mycologia. **51,** 49—51, 1959.
4. COLE, J. O., ROSS, S. & BOUTHILET, L. "Improvement of Clinical Drug Evaluation Reports". Psychopharmacology Frontiers (Edited by N. S. KLINE). Little, Brown & Co., Boston, 1959.
5. STEIN, S. I. "An Unusual Effect From a Species of Mexican Mushrooms, Psilocybe cubensis". *Mycopathologia et Mycologia Applicata.* **9,** 263—268, 1958.
6. HOFFMAN, A., et al. *Experientia.* Vol. **XIV/3,** 107, 1958; ibid. **XIV,** 397, 1958.
7. WASSON, V. P. & RUSSIA, G. R. Mushrooms and History. Pantheon, New York, 1957.

CARATTERISTICHE MORFO-BIOLOGICHE DI CEPPI FUNGINI DEL GENERE *GEOTRICHUM* ISOLATI DA MATERIALE UMANO

G. CARETTA [1])

Centro di Micologia Medica presso l'Istituto Botanico dell 'Università di Pavia (Direttore: Prof. R. CIFERRI)

Istituto di Clinica Medica Generale e di Terapia Clinica dell'Univ. di Pavia (Direttore: Prof. P. INTROZZI)

(31.III.1959)

Sulle caratteristiche morfologico-culturali e biologiche del genere *Geotrichum* e le sue specie si sono scritte parecchie pagine, anche recentemente, e potrebbe sembrare ozioso il ritornarvi sopra ancora se si ammettesse che gli interrogativi posti da questi funghi siano ormai chiariti. In realtà chiunque abbia occasione di isolare più ceppi di Geotrico, rimane perplesso sulla loro variabilità e quindi sui limiti da assegnare a quella che, se non l'unica è certamente la specie di più comune reperimento: il *Geotrichum candidum.* Avendo avuto occasione nel corso di questo ultimo biennio di isolare un certo numero di lieviti e pseudolieviti da pazienti affetti da varie malattie, inevitabilmente abbiamo accumulato anche ceppi di questo genere, il cui riferimento a *G. candidum,* sulla scorta delle notizie tratte da AA. che ci hanno preceduto in questo lavoro, ci ha lasciati perplessi.

Origine dei ceppi

La serie dei ceppi di cui al presente studio e stata isolata da ammalati ricoverati in Clinica Medica e affetti da affezioni diverse, che si supponevano portatori di lieviti in generale e piú particolarmente di *Candidae,* nel corso di ricerche svolte ad altri fini. Premettiamo subito che mai ci é dato osservare un qualsiasi rapporto tra le affezioni, qualunque fossero, degli ammalati e la presenza di Geotrichi. Ció vale particolarmente per gli esami coprologici. I ceppi di cui alla presente contribuzione, in numero di 12, derivano da

---

[1]) Il mio vivo ringraziamento al Prof. R. CIFERRI i cui preziosi consigli e la cui guida mi hanno permesso il completo svolgimento del lavoro.

130 ammalati e soltanto dalle feci: contrariamente a quanto si é riscontrato per i lieviti, nessun Geotrico é stato isolato da sputi o da cavitá polmonari per broncoaspirazione. Soltanto in 3 casi é stato isolato simultaneamente un Geotrico e un lievito, generalmente una *Candida*.

A un primo esame morfo-culturale, i funghi artrosporei sono stati suddivisi in due gruppi: quelli che piú o meno latamente rientrano in *G. candidum*, quale é comunemente inteso dagli studiosi di micologia medica e i lieviti che ne sono variamente aberranti.

In questa prima contribuzione ci soffermeremo brevemente sul I° Gruppo, in quanto soltanto un loro approfondito studio poteva permettere abbordare l'indagine del II° Gruppo.

Oltre questo gruppo di Geotrichi abbiamo tenuti presente, a scopo comparativo, altri ceppi di micoteca di varia origine e provenienza, che circolano sotto il binomio di *G. candidum*, nonché 3 ceppi n.129, 348 e 490 che a nostra richiesta ci sono stati gentilmente inviati dal Prof. A. Chaves Batista, Direttore dell'Istituto di Micologia dell'Universitá di Recife, Pernambuco (Brasile), anche al fine di avere ceppi di Geotrichi di diversa origine geografica.

Dobbiamo subito dire che questi ceppi brasiliani rientrano pienamente nel gruppo di forme che noi consideriamo appartenenti a *G. candidum* e questi ceppi saranno quindi inclusi, allorché ci paia utile, in questo contributo.

Metodi di studio

I metodi di studio sono quelli stessi impiegati dai ricercatori che, piú di recente si sono occupati di questi funghi, e in particolare quelli di Carmichael (1957), che ne é stato il piú recente studioso.

Sebbene la cultura sia agevole in tutti gli usuali mezzi di laboratorio, ci é parso particolarmente utile l'impiego di un mezzo relativamente povero di sostanze nutritive, quale é l'agar al decotto di farina di mais, lo stesso impiegato per provocare la formazione di clamidospore in *C. albicans* in quanto su tale terreno si nota anche per i Geotrichi una maggior omogeneitá morfologica. Poiché varie modalitá di preparazione di questo terreno sono state proposte di recente indichiamo quella da noi usata: a 40 gr. di farina di mais si aggiunga 1 litro d'acqua mescolando bene. Si metta, quindi, il recipiente in termostato a 55—58° C per un'ora quindi si filtri, si agarizzi e si sterilizzi a 122° C. Il vantaggio di questo terreno su quelli piú ricchi in metaboliti sta nello schematizzare, per cosí dire, la morfologia microscopica del fungo, provocando nello stesso tempo, quel tanto di aberrazioni morfologiche compatibili con l'attivitá vitale del fungo.

Ció si rende meglio evidente osservando direttamente lo sviluppo delle colonie in capsule Petri al microscopio e a modesto ingrandimento e, meglio ancora, in culture su portaoggetti con il metodo di Rivalier-Seydel.

Lo strato estremamente sottile di agar nutritizio e forse una certa tendenza ad un disseccamento precoce, rendono ancor piú schematica la morfologia del fungo nelle fasi di moltiplicazione vegetativa. Questa tecnica permette inoltre agevolmente lo studio di dettaglio di certi fatti morfologici al microscopio a contrasto di fase senza le perturbazioni inerenti il fissaggio e la colorazione. Quasi come questo terreno é utile, agli stessi fini, il terreno all'acetato sodico, che é lo stesso impiegato ai fini di provocare la sporificazione di lieviti sporigeni, cosí costituito: acetato sodico anidro 0,3—0,5 % in acqua agarizzata all'1, 5 % a pH = 6,5—7,0. Si sterilizza quindi per 20 minuti primi a 122° C. Questo studio é stato integrato, naturalmente, dall'esame comparativo su terreni ricchi quali l'agar di Sabouraud glucosato. Glucosio gr. 20, peptone gr. 10, agar gr. 15, acqua ml. 1000. Per i terreni liquidi ci siamo limitati allo studio della crescita del fungo studiandone le caratteristiche culturali e morfologiche del velo in acqua pepto-glucosata cosí composta: glucosio gr 20, peptone gr 10, acqua ml. 1000. Non abbiamo insistito su questa ricerca perché non ci é parsa particolarmente utile ai nostri fini.

Per quanto concerne le indagini sulle attivitá biochimiche, sono state eseguite le seguenti ricerche:

1. prove dell' attivitá fermentativa. Le fermentazioni sono state effettuate con la technica di DURHAM in acqua peptonata all'1 % con l'aggiunta dell' 1 % di glucosio, saccarosio, galattosio, lattosio, raffinosio, maltosio;
2. prove di assimilazione dei glucidi e dei nitrati. Le prove di assimilazione sono state giudicate dall' opacitá dei mezzi liquidi di cultura in presenza di nitrato potassico come sola fonte di azoto e dei vari glucidi come sorgente di carbonio. In ció ci siamo attenuti alle tecniche discusse da CIFERRI (1958) e alle norme di LODDER & KREGER VAN RIJ (1952)

Morfologia dei ceppi studiati

Per quanto concerne le colonie giganti non abbiamo potuto mettere in evidenza caratteristiche differenziali di valore ai fini tassonomici; tutti i ceppi ripetono le caratteristiche precedentemente piú volte descritte da vari studiosi e in particolare da CARMICHAEL.

Per quanto si riferisce alla morfologia microscopica e particolarmente alla morfologia dello sviluppo e della fruttificazione vegetativa i 10 ceppi (segnalati col numero romano dall'I al X) sono risultati identici, ed é questa morfologia media che viene descritta in dettaglio. Leggermente diversi sono stati i ceppi etichettati come XI e XII e a continuazione ne indichiamo le caratteristiche differenziali. L'osservazione microscopica viene effettuata dopo 1, 2 e 3 giorni di crescita. Le culture sono montate in lattofenolo e lattofucsina (CARMICHAEL, 1955).

Caratteri culturali.

Terreni solidi:

a) Sabouraud ordinario, temp. 22—25° C.

Inizialmente si ha lo sviluppo di colonie sottilissime, piane, striscianti, ad ife poco evidenti. Successivamente la crescita diventa piú abbondante ed uniforme con formazione di colonie estese, bianche, lisce, talora con un'area centrale rilevata, fiocconosa o spinosa con ife erette di 2—3 mm.

La superficie puó avere un aspetto vellutato o polveroso o cotonoso con anelli concentrici. Rovescio della colonia generalmente liscio con scarsa tendenza ad approfondirsi nel terreno.

La crescita é rapida. Il diametro delle colonie é di 40—60 mm dopo 10 giorni.

b) Agar-mais, temp. 22—25° C. Le colonie sono sottilissime con aspetto quasi pelliculoso, incolore, estese con margine indistinto; é visibile la crescita raggiata del micelio.

Crescita rapida: il diametro delle colonie é di 15—35 mm dopo 5 giorni, 30—60 mm dopo 10 giorni.

c) Acetato sodico, temp. 22—25° C. Le colonie presentano l'aspetto macroscopico della crescita su agar-mais senza modificazioni sensibili delle caratteristiche suindicate.

Terreni liquidi:

Acqua peptoglucosata: formazione iniziale di un velo superficiale d'aspetto polveroso uniforme, liscio od appena ondulato o cotonoso con formazione di anello incompleto e ad isolotti. Frequentemente alcune colonie presentano un velo con aspetto a piastrelle esagonali.

Dopo 5 giorni la crescita é superficiale e profonda. La colonia é bianca, spessa con aspetto polveroso o cotonoso. Il liquido rimane trasparente ma ha in sospensione sottili fiocchi uniformi di micelio che a poco a poco si depositano sul fondo. La reazione é acida, senza formazione di gas.

Caratteri morfologici.

Ceppo I (*Sabouraud ordinario*):

Da un tallo basale a doppia parete ora ovoidale del diam. di 10μ circa, ora rettangolare ad angoli arrotondati delle dimensioni di 12 × 7, 2 μ ,si diparte un micelio settato piú o meno differenziato che si ramifica in larghe ife primarie generalmente molto lunghe da cui hanno origine rami laterali a ramificazione pseudodicotomica composte da segmenti artrosporei a contenuto citoplasmatico omogeneo.

L'ifa primaria é composta da tratti miceliari jalini a contenuto citoplasmatico indifferenziato (24 × 3,6; 45, 6 × 4, 8 μ) e da tratti miceliari piú corti a contenuto citoplasmatico omogeneo e opaco (7, 2 × 4, 8; 14, 4 × 7, 2 μ). Tav. I, C), D). Tav. II. A).

I tratti miceliari con protoplasma jalino trasparente, sono rettangolari con pareti laterali talora ondulate o ricurve: in corrispondenza della settazione gli angoli sono retti e quindi i setti lineari allorché il successivo tratto é pure jalino; arrotondati o smussati con accenno a concavitá dell' estremitá articolare allorché il successivo tratto miceliare é opaco.

I tratti miceliari opachi sono intercalati in numero di 3—7 e piú ad ogni tratto miceliare jalino. (Tav. II, A.) La loro forma, inizialmente rettangolare va successivamente assumendo un aspetto cuboideo; presentano angoli arrotondati e membrana ben evidente e spessa. Il loro numero é limitato a 3—5 nella parte basale dell'ifa, intercalati da tratti miceliari jalini mentre, nella parte distale, si susseguono in numero notevole rispetto ai tratti miceliari jalini, tanto da comporre quasi completamente alcuni rami laterali e alcuni tratti distali dell'ifa primaria.

Le catene laterali possono avere origine sia da tratti miceliari jalini che da tratti miceliari opachi piú frequentemente hanno origine dalla parte distale di un tratto miceliare opaco. (Tav. I D) Tav. II, E). F)). Sia i tratti miceliari opachi che jalini presentano all'estremitá distale, che é origine del ramo laterale, o una fase pseudogemmante lateralmente in cui li citoplasma del tratto miceliare si continua per un breve tratto nel ramo laterale dell'ifa, o una fase settata con formazione di una parete articolare laterale. Le artrospore dei rami laterali sono inizialmente rettangolari e possono assumere successivamente nella parte distale un aspetto cuboideo. I rami laterali sono composti da 18 fino 50 artrospore di 9, 6 × 3, 6; 4, 8 × 2,4 μ; lungo le catene laterali sono intercalati alcuni cellarioli ovoidali e liminoformi.

Le ife possono raggiungere la lunghezza di 0,5 mm. I tratti miceliari si formano in catene per disarticolazione basipeta su setti delle ife principali. In corrispondenza della settazione si osserva una tendenza alla concavitá della parte distale che ben si adatta alla convessitá della parte prossimale del tratto miceliare successivo (tipo articolazione ossea) con accenno a rigonfiamento delle estremitá distali e prossimali tanto da avere l'impressione della presenza di un menisco.

Talora l'estremitá distale di un tratto miceliare jalino presenta due faccette articolari laterali su cui si articolano i tratti miceliari iniziali dei rami laterali.

Frequenti sono nella parte distale delle ife principali le ramificazioni a forcella con bracci della lunghezza di 40 μ circa: tale tipo di ramificazione é formato da segmenti miceliari jalini.

Il tallo basale contiene un nucleo e il suo citoplasma puó continuarsi per un tratto lungo l'ifa vegetativa.

Agar-mais:

L'osservazione microscopica della cultura mette in evidenza coloniette scarsamente ramose e con rami laterali brevi a sviluppo notevolmente ridotto nei confronti di quelle dello stesso ceppo coltivato su Sabouraud. Il diametro dell'ifa principale é di 2,4 μ circa (quindi nel rapporto 1—2 rispetto al diametro delle culture dello stesso ceppo su Sabouraud) e la lunghezza di 364 μ circa (rapporto 1—2).

Le ife a carattere artrosporeo si originano da talli basali ovoidali (diam. 4,8 μ) rettangolari (12 × 7, 2 μ). (Tav. I. E) I talli basali presentano una capsula spessa, talora é visibile un nucleo; il citoplasma é granuloso e si continua per un breve tratto nella parte iniziale dell'ifa. Da ogni singolo tallo possono avere origine una o piú ife. In genere le singole ife hanno origine dai talli rettangolari in corrispondenza degli angoli omologhi ed eterologhi.

L'ifa é pure composta da tratti miceliari jalini (19, 2 × 50,2) × 2,4 μ con citoplasma talora d'aspetto granuloso e da tratti miceliari opachi (7, 2 × 2,4) (26, 4 × 2, 4) μ con citoplasma omogeneo. (Tav. II. B). L'ifa puó essere breve e formata solo da tratti miceliari opachi. I tratti miceliari dell'ifa principale sia jalini che opachi sono rettangolari ad angoli retti con estremitá piuttosto rigonfiate. I singoli tratti miceliari si generano per frammentazione centrifuga (basipeta) delle ife; in corrispondenza della settazione l'estremitá distale di ogni tratto miceliare presenta come una concavitá che si adatta alla convessitá dell'artrospora successiva: i margini interni sono spessi, jalini, assumendo come l'aspetto di un cercine. In genere la parte terminale dell'ifa é composta da tratti miceliari opachi quasi staccati (7, 2 × 2, 4 μ).

I rami laterali originano pure dalla parte distale di un tratto miceliare sia jalino che opaco con le stesse modalitá descritte per le culture su Sabouraud. I rami laterali sono piú corti e composti in media da 8—13 artrospore generalmente opache (7,2 × 2, 4; 4, 8 × 2,4 μ) in cui la disarticolazione tra le singole artrospore presenta molto meno evidenti le caratteristiche descritte per l'ifa principale. La forma delle artrospore é rettangolare con angoli inizialmente retti e successivamente smussati. Lungo i rami laterali sono intercalati alcuni cellarioli ovoidali e limoniformi.

I preparati montati in lattofucsina evidenziano meglio le caratteristiche morfologiche dei tratti miceliari jalini e opachi, mentre é meno marcato l'aspetto caratteristico della settazione.

Acetato sodico:

Da un tallo ovoidale a capsula spessa, del diam. di 7,2 μ retangolare (12 × 4,8 μ) si diparte un micelio jalino, ramoso e poco sviluppato settato, formato da tratti miceliari (36 — 16,8) × 4,8 μ jalini, a parete ondulata o ricurva, talora a contenuto citoplasmatico granuloso, e da rari tratti miceliari opachi (9,6 — 14,4) × 3,6 μ. I singoli tratti miceliari si generano per frammentazione centrifuga (basipeta) delle ife; i segmenti miceliari dell'ifa principale presentano, come sugli altri terreni, alle estremitá un rigonfiamento peró piú marcato, dando cosí l'aspetto di una canna di bambú all'ifa. Tale aspetto va scomparendo nella parte terminale dell'ifa formata generalmente da tratti miceliari opachi rettangolari (7,2—12) × 2,4μ. Frequente é l'osservazione di tratti miceliari opachi apicali e no le cui estremitá distali, tozzamente allargate, presentano due o tre faccette su cui si articolano le prime artrospore di rami laterali. I rami laterali originano appena sotto e lateralmente la estremitá distale di un tratto miceliare jalino od opaco. I rami laterali sono composti da 5—12 artrospore rettangolari ad angoli smussati od arrotondati (9, 6—4,8) × 4, 8 μ.

Le ife possono raggiungere la lunghezza di 240 μ.

Ceppo XI: *Sabouraud*:

A differenza del ceppo precedente si nota nella parte basale una prevalenza di tratti miceliari jalini componenti un micelio tortuoso di 5 μ di spessore, ramoso per le frequenti corte ramificazioni laterali. In altri punti i singoli tratti miceliari sono opachi, rettangolari ad angoli smussati od ovoidali (8 × 4 μ) e ammassati: solo perifericamente si dispongono in catene per il susseguirsi di tratti miceliari opachi intercalati da qualche tratto jalino con aspetto di uno pseudomicelio che ha corti rami laterali.

In altri punti del preparato si ha la crescita con le caratteristiche morfologiche descritte per il ceppo I.

La parte terminale peró di ogni ifa, é formata da artrospore ovoidali (6—5) × 4 μ; nella parte apícale sono frequenti artrospore ovoidali a forma di bottiglia o di fiasco provviste di inclusi globosi, piú grosse delle sottostanti, con membrana spessa e bene evidente (12—9) × 6 μ da cui si dipartono da 5 a 7 artrospore rettangolari (7,2—5) × 1,8 μ.

Ceppo XII: *Sabouraud-ordinario*:

Da un tallo basale a doppia parete ovoidale del diam. di 7,2 μ circa si diparte un micelio settato che si ramifica successivamente in ife secondarie da cui hanno origine rami laterali composti da tratti micelici rettangolari a contenuto citoplasmatico omogeneo.

L'ifa primaria é composta da tratti miceliari jalini (31,2—13) × 3, 6 μ e da tratti miceliari piú corti, rettangolari a contenuto citoplasmatico piú omogeneo, opaco (9,6—4,8) × 3,6 μ.

I tratti miceliari jalini sono rettangolari, con settazione ad angolo retto, con pareti talora ondulate o ricurve. I tratti miceliari opachi sono pure rettangolari, talora quadrangolari. Sono intercalati in numero vario da 4 a 10 ad ogni tratto miceliare jalino del ramo principale; talora si susseguono in numero superiore tanto da comporre lunghi tratti dell'ifa principale.

I rami laterali originano secondo le modalitá del ceppo I da tratti miceliari jalini e opachi: sono composti prevalentemente da artrospore opache a configurazione sempre rettangolare in numero da 30 a 60; (7,2—3,6) × 2,4 μ

Le ife possono raggiungere la lunghezza di mm 0,5.

Frequenti sono nella parte distale dell'ifa ramificazioni a forcella.

Agar-mais:

Micelio settato piú o meno sviluppato in ife, originantesi da talli basali, od ovoidali, lunghe fino a 80 μ e 460 μ. L'ifa primaria é composta da tratti miceliari jalini (68—14) × (3—2) μ e da tratti miceliari opachi (8—4) × (3—2) μ rettangolari con settazioni ad angolo retto.

I rami laterali originano indifferentemente da tratti miceliari jalini e opachi e sono composti da 40—60 artrospore opache rettangolari (6—4) × (2—1,5) μ e lunghe fino a 80 μ; talora in un tratto del ramo laterale sono interposte tratti miceliani jalini lunghi fino a 20 μ.

Acetato sodico:

Caratteristiche simili morfologicamente a quelle descritte per il ceppo I.

Ceppo II: caratteri morfologici in Sabouraud, agar-mais, acetato sodico analoghi a quelli descritti per il ceppo I:

Ceppo III = Ceppo II.
Ceppo IV = ,, ,, .
Ceppo V = ,, ,, .
Ceppo VI = ,, ,, .
Ceppo VII = ,, ,, .
Ceppo VIII = ,, ,, .
Ceppo IX = ,, ,, .
Ceppo X = ,, ,, .

Caratteri biochimici.

Fermentazione: i risultati sono stati uniforme in tutti i ceppi studiati.

Glucide	*Gas*	*Acido*
Glucosio	—	+
Galattosio	—	+
Saccarosio	—	—
Lattosio	—	—
Raffinosio	—	—
Maltosio	—	—

Solo il ceppo VIII ha avuto la fermentazione positiva per il glucosio e il galattosio.

Assimilazione degli idrati di carbonio:

	Ceppi:											
Glucide	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XI
Glucosio	++	++	++	++	++	++	++	++	++	++	++	++
Saccarosio	+	+	—	—	+	++	+	+	—	—	+	+
Galattosio	++	++	++	++	++	++	++	++	+	+	++	++
Lattosio	—	—	—	—	—	—	+	+	—	—	—	—
Raffinosio	+	—	+	—	—	—	—	+	+	—	—	+
Maltosio	—	—	±	+	±	—	+	±	+	+	+	+
Sostanze azotate:												
Peptone	+++	++	++	++	++	+++	++	++	++	++	++	++
KNO_3	±	+	±	±	+	±	±	±	±	±	±	±

DISCUSSIONE DEI RISULTATI

L'insieme dei ceppi da noi indagati, pure nella loro variabilitá individuale, manifestano una notevole omogeneitá sia morfologica che culturale e biochimica, e debbono essere riferiti ad una sola specie, che corrisponde a quella comunemente intesa quale *Geotrichum candidum*. Desideriamo peró segnalare alcuni fatti che non sono stati osservati dagli autori che ci hanno preceduto (BERKHOUT, 1923; LOUBIERE, 1924; CIFERRI & REDAELLI, 1929; DODGE, 1936; COLONNELLO, 1944; BOBROV, 1951; WINDISCH, 1953; CARMICHAEL, 1957) o ve ne sono fuggevoli e non chiari accenni.

Uno degli aspetti é un dimorfismo del micelio che si riflette anche in variabilitá delle artrospore. Fermo restando che questi organi di moltiplicazione si producono soltanto per scissione delle ife miceliche in segmenti, ne possiamo chiarire l'origine e l'evoluzione.

Lo sviluppo della futura colonia ha origine non da qualsiasi artrospora, ma da quelle peculiari corte e tozze cellule non jaline, che rappresentano, a nostro giudizio, organi intermediari tra le clamidospore e le artrospore usuali che denomineremmo artrospore clamidosporioidi, (Tav. I. C), per le loro caratteristiche di densitá e opacitá del protoplasma, spessore e duplicitá della parete esterna, forma raccorciata in relazione allo spessore per cui la lunghezza tocca appena il doppio del diametro, mentre il diametro stesso é due o tre volte quello delle artrospore ordinarie. Il carattere clamidosporioide di queste artrospore é accentuato dalla duplicitá della tunica, non sempre evidente, ma visibile sopratutto alla loro germinazione, che si ha per una estrusione dell' endosporio, come fanno le vere clamidospore e molte spore sessuate di altri funghi. Artrospore clamidosporioidi sono riconoscibili nella fig. 9 del lavoro di CARMICHAEL (1957) mentre nella fig. 8 sono riconoscibili artrospore comuni.

Dalla germinazione di queste artrospore clamidosporioidi ha origine il micelio che potrá frammentarsi o meno in artrospore.

Questa osservazione pone l'interessante problema della funzione delle ben piú numerose artrospore usuali (non clamidosporioidi).

Su questo punto non sapremmo pronunciarci. Sta di fatto che una germinazione quale osservata nelle artrospore clamidosporioidi non é mai stata notata nelle artrospore usuali, che dovrebbero quindi essere considerate come semplici ramuscoli abbreviati di micelio. In altri termini l'apparente funzione moltiplicativa delle artrospore ordinarie quale é stata considerata da tutti gli AA. che precedentemente si sono occupati di queste specie, non corrisponde affatto alla realtá: queste presunte artrospore non paiono essere organi di moltiplicazione, ma rappresentano lo stadio finale dell'evoluzione maturativa dei rami miceliali.

Le profonde ragioni del perché avviene questa fissione ci sfuggono, per ora almeno.

D'altro canto non tutti i segmenti dei rami micelici sono atti a ramificare, ed eventualmente essere quindi sede di fissione.

I rami laterali originano preferenzialmente dall'estremitá distale di cellule del micelio nel quale si addensa il protoplasma dando l'apparenza di un segmento, quando in realtá é solo la prosecuzione dell'ifa micelica. Una volta sviluppatosi il ramo micelico, puó essere sede di fissione o permanere non scisso. In ogni caso la fissione procede con notevole rapiditá piú spesso dalla base verso l'estremitá distale, potendo interessare sia una parte, che piú parti o tutto il ramo, cioé il micelio in fissione alternantesi a tratti indivisi. In qualche caso la fissione sembra procedere in senso opposto o addirittura nei due sensi, ma trattandosi di un fenomeno che é difficile seguire al microscopio, non sapremmo pronunciarci definitivamente in merito.

In ogni caso tutti i rami miceliari sono potenzialmente sede di fissione, senza che possano individuarsi, morfologicamente almeno, in settori specializzati per la fissione o la continuitá. Il meccanismo di formazione delle artrospore ci é parso essere il seguente. Mentre dove l'ifa non é sede del processo di scissione il protoplasma é distribuito omogeneamente nel lume dell'ifa, in una fase che possiamo dire di pre-artrosporulazione, si delineano dei falsi setti che sono dati semplicemente da zone trasversali irregolari di minor addensamento del plasma, o, di converso queste strie trasversali chiare sono limitate da due addensamenti di plasma. E' questa fase che in una schematizzazione approssimativa puó essere rappresentata (ed é stata piú volte disegnata) come settazione.

Successivamente la zona chiara di separazione del plasma si delinea piú nitidamente perché le due estremitá plasmatiche sono sede di precipitazione di una sostanza rifrangente che supponiamo di natura callosica. In tal modo lo pseudo setto che inizialmente spiccava in chiaro nel protoplasma limitante piú addensato e quindi piú scuro, diviene opaco poiché la precipitazione delle sostanze che costituiranno i due apici della tunica involucrale dell'artrospora sono piú rifrangenti. Questa precipitazione significa anche interruzione della continuitá protoplasmatica. In tal modo il protoplasma interstiziale e il residuo della tunica miceliale continua primitiva va rapidamente in lisi individualizzando cosí le artrospore in una catenella diritta o che segue le sinuositá del ramo miceliare che diede loro origine.

Poiché l'ifa generatrice puó crescere basalmente, la catenella delle artrospore viene eventualmente distinta dal basso verso l'alto. A ció si deve il fatto che alcune artrospore, scivolando sul substrato di cultura si dispongano piú o meno trasversalmente dando origine a catenelle angolose a zig-zag o disordinate e persino ad aggregati capoliniformi di artrospore.

L'ulteriore morfologia di queste artrospore ormai costituenti entitá individualmente distinte, varia probabilmente con fattori d'ordine fisico chimico di cui possiamo supporre la natura.

Poiché l'estremitá delle artrospore ha una tunica notevolmente piú spessa della tunica laterale, tende giá per suo conto ad una smussatura degli angoli, cioé ad assumere la forma cuboidea, linea arrotondata o parallelepipeda arrotondata. Ma se il mezzo esterno é piú concentrato del mezzo protoplasmatico o interno deve aver

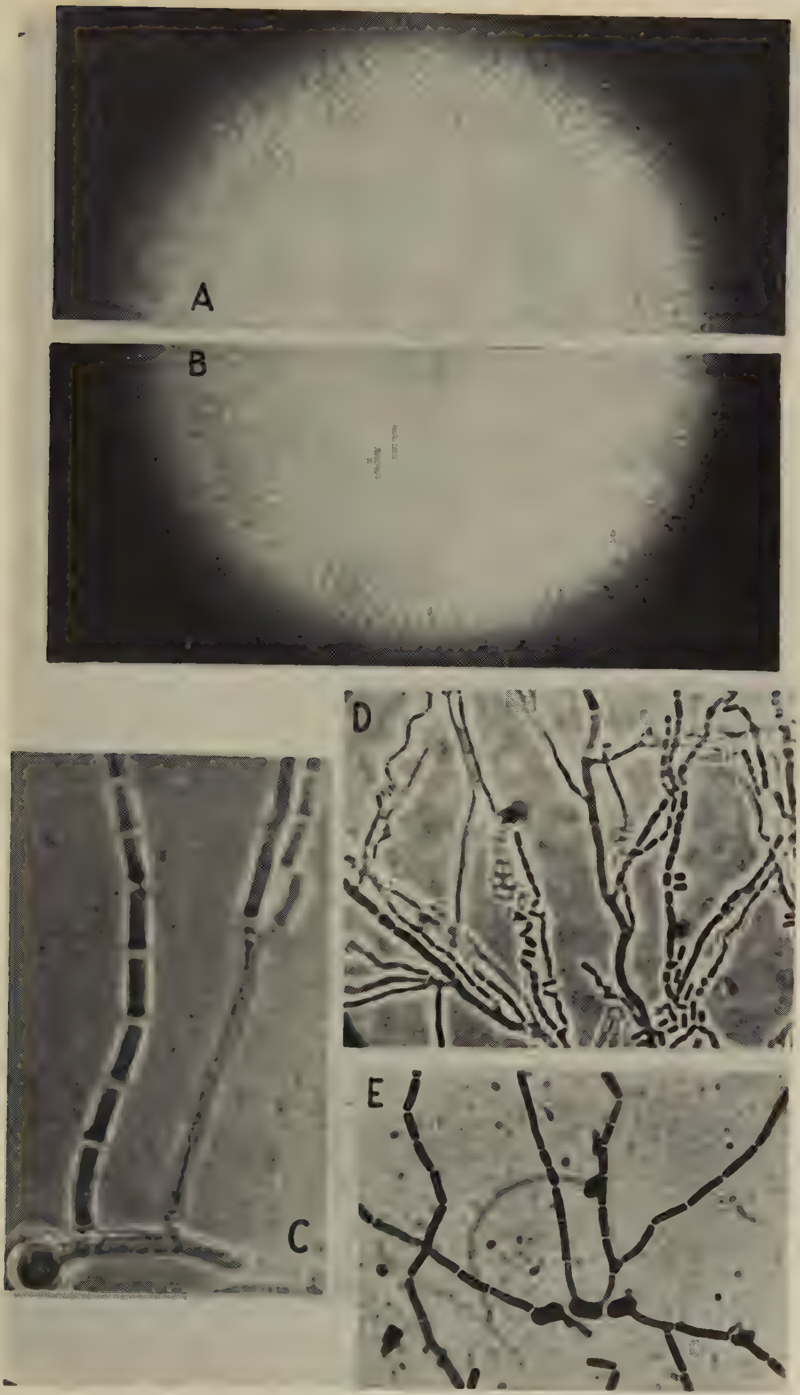


Tavola I

A, B). Colonie giganti su agar Sabouraud mostranti le minime differenze fra i varii ceppi

C). Artrospora clamidosporioide germinante su agar mais e tratti miceliari ialini e opachi. (X ca. 1200).

D). Tratti miceliari ialini e opachi e loro ramificazioni su agar Sabouraud (X ca. 300).

E). Artrospore clamidosporioidi germinanti su agar mais (X ca. 500).

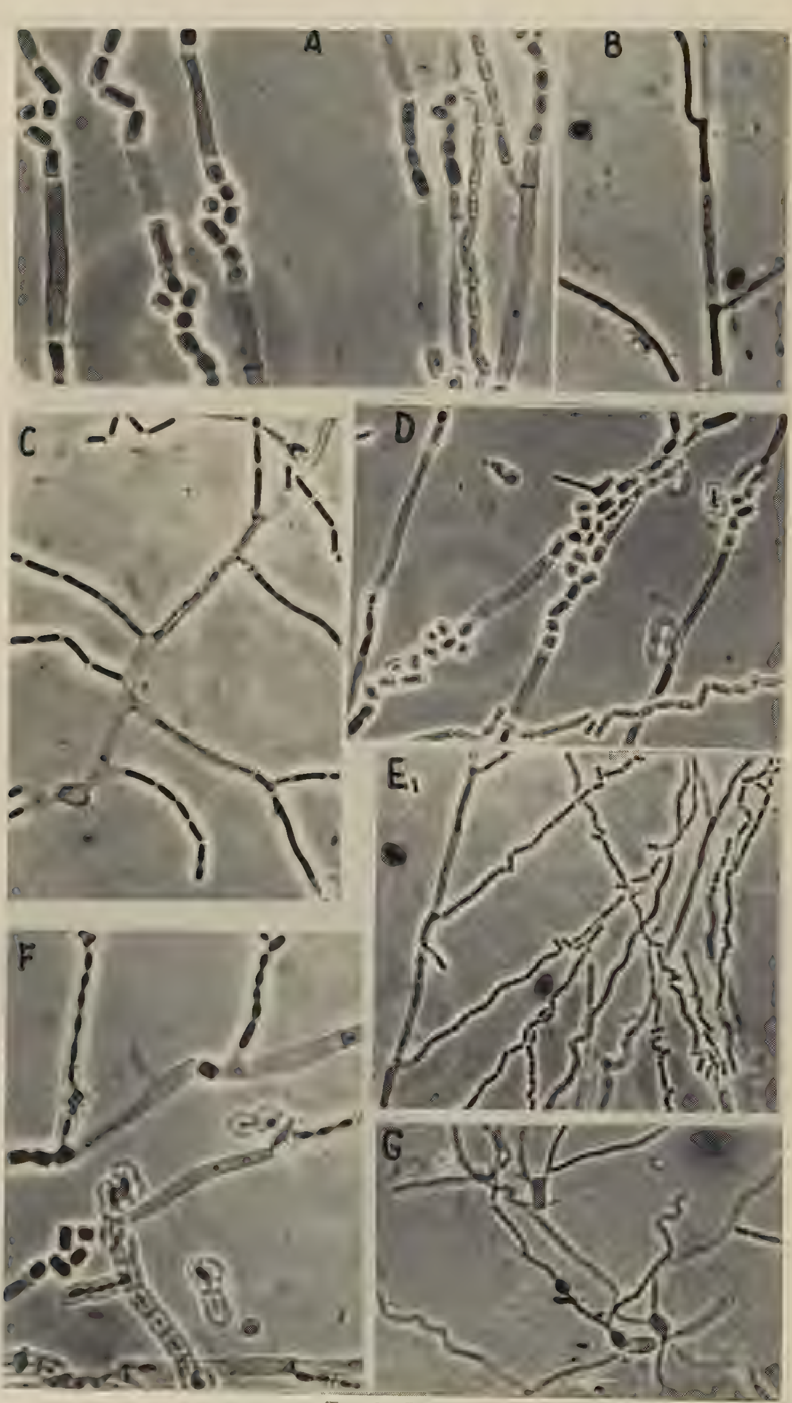


Tavola II

A, B, C, D, E, F). Tutte le fotografie evidenziano l'alternarsi dei tratti miceliari ialini e opachi e loro ramificazioni:

A, D, F). Su agar Sabouraud (X ca. 500).

B), su agar-mais (X ca. 500). C) idem (X ca. 300).

E), su agar-Sabouraud (X ca. 300).

G) Artrospore Clamidosporioidi germinanti su agar-mais. (X ca. 300).

luogo un fatto di osmosi, con fuoriuscita d'acqua o di soluzioni, che portano ad una certa contrazione dell'artrospora. Questa contrazione si riflette sulle pareti laterali, che come si é detto sono quelle sottili, mentre rimangono all'incirca integre le pareti distali e basali piú spesse. La conclusione é che la forma che assume individualmente l'artrospora é a doppia mazza di tamburo o a tibia, e se le artrospore sono tuttora seriate, l'aspetto a canna di bambú. Qualcosa di simile deve aver osservato GOIDÀNICH (1935), a giudicare dalla fig. 2 del *Geotrichum doliiforme*, il quale peró non é riferibile al gruppo di Geotrichi che qui c'interessa. Infatti egli scrive "che le catene dei conidi sembrano rivestite da una pellicola. I conidi, infatti, possono essere piú o meno distanziati l'uno dall'altro senza, con questo, liberarsi. In quel tratto che li separa esiste una sottilissima pellicola jalina . . ." corrispondente a quello che abbiamo chiamato protoplasma interstiziale residuo.

Tutto quanto si é detto sinora, riflette le artrospore comuni che non abbiamo visto mai germinare e non quelle che abbiamo indicato come artrospore clamidosporioidi, che sono sede di fatti germinativi. Qui la precipitazione dei costituenti la membrana di nuova formazione é all'incirca omogenea per tutto il tallo, cioé non si hanno evidenti differenze di spessore tra le due estremitá e i lati (viste in sezione ottica). A questo notevole e relativamente uniforme spessore si deve il fatto che tali artrospore clamidosporioidi assumano una forma cuboidea ovalata e persino elittica o sferoidea. Né, in virtú dello spessore, paiono essere sede di fenomeni di esosmosi. All'addensamento del protoplasma e allo spessore delle pareti si deve pure la relativa opacitá di questi elementi, in confronto alle comuni artrospore, per cui abbiam oparlato di elementi relativamente opachi.

Il fenomeno di irregolare distribuzione del contenuto plasmatico ha luogo anche nelle ife miceliche che non si divideranno per scissione. E'del resto questo un fatto comune negli ifomiceti, ma forse piú frequente in *Grotrichum* che in altri generi di funghi. Il protoplasma migra in parte negli apici di crescita ove, attraverso le cribrature dei setti, migrano pure i nuclei. Tale passaggio del protoplasma puó essere parziale, ed allora la cellula sottostante l'apice di crescita appare a protoplasma meno denso e vaccuolato ma tuttora evidente; oppure la cellula sottostante puó svuotarsi di citoplasma quasi completamente ed apparire del tutto o quasi del tutto vuota, cioé é ridotta alla sola membrana esterna e ai setti divisori. E' evidente pertanto come questa cellula appaia al microscopio a contrasto di fase come otticamente vuota, anche per contrasto con l'opacitá delle celllule dense di plasma. E' evidente che queste cellule senza plasma non sono sede di fissione o di ramificazione.

TASSONOMIA

E' ormai generalmente accettato che i Geotrichi nulla hanno a che vedere con ilieviti in seno o in appendice ai quali erano posti dagli studiosi meno recenti di micologia zimologica e medica. Infatti le trattazioni piú recenti sui lieviti (ad es. la monografia di LODDER & KREGER VAN RIJ, 1952) non ne fanno menzione. La ragione sta nel fatto che i lieviti hanno una forma di moltiplicazione per gemmazione (salvo il genere sporigeno *Schizosaccharomyces* e il corrispondente genere anascosporeo *Schizotorulopsis* CIF. 1930). I Geotrichi invece non gemmano mai, ma si riproducono per frammentazione di micelio o artrosporulazione.

Ne consegue quindi implicitamente l'accettazione della famiglia Geotrichaceae CIF. (1955; 1957) che sostituisce il termine di taxon artrosporeae tradizionale a partire da GUÉGUEN ma non conforme alle regole internazionali di nomenclatura botanica (in merito vedi pure CIFERRI & REDAELLI, 1934).

Nel suo Manuale di Micologia Medica (1958) il CIFERRI cosí diagnostica questa famiglia:,,caratterizzata da micelio vero, ramoso, jalino e settato, che si scinde, in parte o del tutto, in frammenti od artrospore, dall'apice verso la base, raramente viceversa, oppure nello stesso tempo con andamento centripeto e centrifugo. Non ha clamidospore e neppure mai blastospore".

Cosí definita, la famiglia include tra i funghi artrosporei provvisti di micelio il solo genere *Geotrichum* LINK (1809) emend. CIF. et RED. (1929) (1). Le asserzioni di WINDISCH. (1953) circa la corrispondenza di forme ascogene del genere *Geotrichum* quale stadio sessuato di *Geotrichum* non é stata mai confermata da nessun studioso, noi stessi inclusi, e non puó accettarsi, sino a prova contraria. Escluderemmo egualmente dal gen. *Geotrichum* le forme a cespituli colorati in rosso o aranciato od ocra, per lo piú un tempo ascritte al gen. *Oospora*.

Una parte, almeno, delle suddette forme, secondo CIFERRI (1943) é riferibile a *Coprotrichum* BONORDEN (1831) quale tipificato da *C purpurascens*. ²) Ció ci permette di escludere dal novero delle specie che qui ci interessano il *Geotrichum doliiforme* GOID. (1935) a cespituli rosso ocraceo e polverosi. Malgrado il fatto che, come si dirá piú oltre, indubbiamente vi sono termini di passaggio tra il *Geotrichum* e *Sporendonema ligneospora*. Uno di questi termini di passaggio é o puó essere il *Geotrichum amycelium* CIF. et RED. (1935) che infatti WINDISCH (1953) riferisce al gen. *Oospora* come *O. amycelica* (CIF. et RED.) WINDISCH (3).

---

¹) Infatti *Schizotorulopsis* non ha né micelio, né pseudomicelio, ed inoltre gli corrisponde il genere ascogeno *Schizosaccharomyces*. *Mauginiella* CAV. (1926) dopo lo studio di ceppi autentici, é stato da CIFERRI (1958) riferito a *Sporendonema epizoum* CORDA (o *Sporendonema sebi* FRIES).

²) Rimane incerta la posizione sistematica delle forme a cespituli grigiastri, di cui al *Coprotrichum cinereum* BONORDEN (1831), riportato da SACCARDO al *Geotrichum cinereum* SACC. (1886).

³) GOIDANICH (1935) richiama l'attenzione sulle affinitá di *Geotrichum* con *Malbranchea* SACC. (1882), facendo peró notare che *Geotrichum* non ha conidi endogeni. Il problema delle affinitá dei funghi a conidi endogeni e pseudoendogeni, é complesso ed esula dall'ambito del nostro studio. Per il genere *Malbranchea* ci riferiamo agli studi di BALDACCI, CIFERRI e VACCARI. (1939) su *Malbranchea*; di RED. e CIF. (1934) su *Sporendonema* (Hemispora VUILL., 1906) nonché a *Nascimentoa* CIF. et BAT. (1956). In merito alla fam. *Chalaraceae* SACC. (1886) che comprende *Chalara mycoderma* BON. e vari altri generi incluso *Malbranchea* vedi Manuale di CIFERRI, giá citato.

Rimane ancora il probleme delle affinitá di *Geotrichum* con *Coremiella.* Questo genere é stato descritto da BUBÁK e KRIEGER (in BUBÁK, 1912) con la specie *C. cystopoides* poco nota, e riconvalidato da MASON (in HUGHES, 1953) con *C. ulmariae,* che viene descritta e figurata. E' caratterizzata da conidiofori in cespituli, ognuno dei quali cresce apicalmente e rampica irregolarmente, talvolta dicontinuamente sino ad essere arborescente verso gli apici. I setti trasversali si formano in successione basipeta nei rami superiori e quelli vicini o in cellule alterne sviluppa pareti ispessite, un processo che pure si effettua in maniera basipeta. Le cellule intermedie perdono il loro contenuto interamente e le pareti laterali rimangono sottili, collassano all'indentro e finalmente si suddividono con facilitá in conidi oblunghi o cubici. Il conidio che si realizzerá si sviluppa dentro la parete esterna dell'ifa originale, e una caratteristica papilla e un sottile poro nelle pareti terminali sono chiaramente visibili. I conidi che si liberano mostrano una minuta dentellatura ad ogni estremitá, che é il residuo della parete esterna dell'ifa originale che diverrá una delle cellule separate. Soltante i rami terminali dei conidiofori si trasformano in conidi. Secondo HUGHES, in *Geotrichum* sp., delle ife ialine semplici o ramose sviluppano setti in successione basipeta; ogni cellula assume pareti ispessite e secede per scissione della parete esterna e per separazione della parete trasversale che presumibilmente ha struttura duplice. I conidi sono jalini e oblunghi a maturitá ma gialli in massa in questo ceppo particolare. L'A.si riporta a LANGERON (1945) per il *G. candidum* e ad ARNAUD & BARTHELET (1936) per i. *G. purpurascens* (BON.) SACC., specie che producono conidi alla maniera di *Geotrichum.* Questi due generi (assieme a *Oidiodendron griseum* ROBAK, 1932), alle forme ,,oidiche'' di basidiomiceti superiori, nonché di *Actinomiceti,* sono inclusi nella sua sezione VII, caratterizzata da specie i cui conidi si genera per settazione e fissione di ife semplici o ramose. Sostituendo il nome di artrospore a conidi nel senso impiegato da HUGHES, é evidente che la sezione VII nella classificazione da lui stabilita si riporta interamente alla famiglia Geotricaceae.

Piú recentemente TUBAKI (1958) ha ripreso la stessa suddivisione, precisando che questi particolari conidi sono detti artrospore, e inserendovi anche il *Geotrichum suaveolens* (LINDER) CIF. Giustamente egli precisa che *Trichosporon* é affine a *Geotrichum* per le artrospore, ma produce anche cellule gemmanti, ed infatti il CIFERRI (1950, 1956, 1958), ne fa una famiglia a parte intermediaria tra i lieviti asporigeni (Torulopsidaceae) e le Geotrichaceae che denomina Trichosporonaceae NANNIZZI (1934) emend. CIF. et RED. (1935).

Secondo TUBAKI anche *Oospora* e *Oidium* (che egli intende nel senso di LINDER, 1942) sono da riferire a questa stessa sezione, in quanto producono artrospore, ma il meccanismo di formazione é diverso. Secondo la modalitá di formazione esogena, nelle ife aeree, si formano setti trasversali, e la parete esterna e il protoplasma delle ife si scindono per formazione; i conidi si sviluppano endogenamente dentro la parete esterna delle ife e una papilla o un minuto poro é chiaramente visibile a ogni estremitá del conidio. Quando i conidi si sono liberati rimangono visibili minute dentellature a entrambe le estremitá che rappresentano i residui della parete esterna dell'ifa.

In conseguenza di ció egli suddivide la sezione VII in due sottosezioni che designa come VII A e VII B. Nella prima include i generi *Geotrichum* anche nelle forme colorate che CIFERRI classifica come *Coprotrichum*, gli *Oidiodendron* e alcune *Oosporae*, mentre nella sezione VII B (quella a formazione endogena di artrospore) pone *Coremiella ulmariae* quale descritta da HUGHES. Egli termina la sua illustrazione dicendo che saranno necessarie ulteriori ricerche su un maggior numero di funghi dimostranti questi tipi di sviluppo.

Anticipando quanto si dirá in un successivo studio su altri ceppi, i Geotrichi da noi studiati sono da riferire al gruppo VII A, cioé alla famiglia Geotrichaceae in senso stretto.[1] Al gruppo VII B appartengono specie Geotricosimili che mostrano le caratteristiche di *Coremiella*, e del quale forse la specie meglio nota tra quelle piú autentiche é *Oospora cuboidea*.

In conclusione vi sono due modalitá di formazione delle artrospore una é esogena e l'individualizzazione delle future artrospore si ha per una frammentarione del protoplasma interifale, e successsiva emergenza di pareti trasversali, mentre la parete longitudinale é quella stessa dell'ifa generatrice. Sono questi i ceppi di *Geotrichum* da noi studiati. L'altra modalitá é quella di individualizzazione delle artrospore in maniera endogena con assunzione di una parete propria anche longitudinale che si accolla alla parete dell'ifa madre. E' questo il tipo *Coremiella*. Per quanto concerne una suddivisione sottogenerica di *Geotrichum* LINK ex PERS., emend CIF. et RED. (1929), CIFERRI (1950, 1956) considera i seguenti sottogeneri:

A) micelio poco sviluppato e di regola assente alla maturitá del fungo, cosicché tutte le ife producono artrospore per scissione. E' questo il sottogenere *Berkhoutia* CIF., VER., et SAG., (1938).
AA) Micelio ben sviluppato, le cui ife solo in parte trasformano in artrospore . . . . B
B) Funghi producenti sostanze ad odore etereo, fortemente odorifere. E'questo il sottogenere *Sachsiella* CIF. (1950—1956), che sostituisce *Sachsia* BAY (1894) quale definito da LINDNER (1898) ed emend. da CIF. et RED. (1922).
La sostituzione di *Sachsiella* a *Sachsia* si deve al fatto che prima di *Sachsia* BAY (1894) era stato descritto un genere *Sachsia* GRISEB. (1860) valido e cronologicamente piú vecchio per un genere di

---

[1] *Geotrichella* ARNAUD (1953), secondo noi, é da riferire a *Geotrichum*. Il nome del nuovo genere fu creato da ARNAUD e classificato fra le Dematiaceae (?) amerospore a conidiofori disarticolabili, in quanto le ife sono formate da un'alternanza di cellule brune e di cellule incolori, aspetto morfologico che noi abbiamo rilevato analogamente in *Geotrichum*.

fanerogame cubane. In questo sottogenere CIFERRI pone soltanto il *Geotrichum suaveolens* (LINDN) CIF. in DIDD. et LODD. (1942) basato su *Sachsia suaveolens* LINDNER (1899). Tale descrizione ha quindi una base effettivamente biochimica.

BB) Che non produce sostanze eteree non odorifere. Sottogenere *Eugeotrichum* CIF. VER. et SAG. (1938). In questo sottogenere si situa il *G. candidum* LINK e le specie che vi gravitano attorno. Non vi sono ragioni per non accettare questa suddivisione sottogenerica, anche se non vi sono segnalazioni recenti a nostra conoscenza, di reisolamento del *G. amycelicum* (specie del resto ammessa anche da WINDISCH, sebbene emendata; con i sinonimi di *Oospora* (*oidium*) gruppo II di RITTER (1930); *Oospora* gruppo M di SEPPERS (1936) e *Oospora amycelica* LEMBKE-DELITSCH (1943) e *Geotrichum suaveolens*, per la cui piú complicata sinonimia rimandiamo alla discussione che ne fanno DIDDEMS & LODDER (1942). Non posiamo dunque aderire alle idee di CARMICHAEL (1957) il quale nel suo d'altronde eccellente studio amplia non solo il concetto di *Geotrichum*, ma la stessa specie *Geotrichum candidum* LINK ex PERSOON (1822), al punto di includervi il *Coprotrichum purpurascens* BON. (1851) e il *Coprotrichum cinereum* BON. (1851) nonché l'*Oidium matalense* CAST. (1915). ¹)

Per quanto si riferisce alla specie, non crediamo che in base alle nostre ricerche i nostri ceppi possono essere riferiti a piú specie. Come si é detto la loro morfologia e il biochimismo sono notevolmente omogenei e sembrano riportarsi tutti al solo *G. candidum*. COLONNELLO (1944) ha portato una lista di 28 binomi con *Geotrichum*, per lo piú (23 binomi su 28) da riferire a generi diversi da *Geotrichum*; quattro da riportare a *Trichosporon* e uno a *G. candidum*. Dal 1944 ad oggi sono state descritte ben poche altre specie di *Geotrichum*, oltre quelle riesaminate o descritte da WINDISCH che appaiono male decifrabili senza un nuovo studio comparativo. Qualche specie come *Geotrichum javanense* VER. (1933) é discriminabile su basi biochimiche piú che morfologiche poiché LUCHETTI (1934) ha dimostrato che queste specie produce alcool etilico a partire dal lattosio.

Crediamo quindi giustificato mantenere il binomio *Geotrichum candidum* per il gruppo di ceppi omogeneo dal punto di vista morfoculturale e fisiologico da noi studiato, senza l'eccessivo spezzettamento del WINDISCH né l'eccessivo ampliamento di CARMICHAEL. In conseguenza al nostro giudizio non é accettabile l'emendamento che questo A. ha proposto per *G. candidum*. A nostro giudizio il fatto

---

¹) Questa specie fu studiata nel 1930 da CIFERRI e classificata dallo stesso nel 1955 come *Trichosporon matalense* (CAST.) CIF. in quanto nel ceppo da lui studiato, e presumibilmente proveniente dell'originale di CASTELLANI, si avevano blastospore ed artrospore. Nel ceppo di CARMICHAEL sarebbero presenti solo artrospore, dal che una delle due ipotesi: o non si tratta dello stesso ceppo studiato dal CIFERRI e la denominazione di *Oidium matalense* nel ceppo CARMICHAEL benché dichiarata proveniente da CASTELLANI é ingiustificata, oppure il ceppo di CASTELLANI vecchio di oltre 40 anni, ha perso la facoltá di formare blastospore, ed é restata soltanto la forma moltiplicativa artrosporea.

che ci sembra degno di maggior rilievo é l'accertamento del significato di quelle artrospore che abbiamo chiamate clamidosporioidi, e alle quali CARMICHAEL pur avendole fotografata non sembra assegnare alcun particolare significato e che invece sono, almeno nei nostri ceppi, i soli organi sede di moltiplicazione vegetativa per formazione di promicelio. Alle piú abbondanti e usualmente descritte artrospore seriate non clamidosporieae non siamo riusciti ad assegnare alcuna evidente funzione nel ciclo vitale del fungo; in particolare non sono sede di fatti germinativi o moltiplicativi di alcun genere.

RIASSUNTO

Dodici ceppi del genere *Geotrichum* isolati da feci umane sono stati studiati nelle loro caratteristiche morfo-biologiche. L'insieme dei ceppi, pur nella loro variabilità individuale presentano una notevole omogeneità sia morfologica che culturale e biochimica. Tale omogeneità morfologica si nota sopratutto in culture su agar mais.

L'autore descrive alcune caratteristiche morfologiche non chiaramente descritte precedentemente da vari autori.

Lo sviluppo della futura colonia ha origine da peculiari artrospore, corte e tozze cellule, non ialine, che l'A. denomina artrospore clamidosporioidi per le loro caratteristiche di densità e opacità del protoplasma, spessore e duplicità della parete esterna, visibile soprattutto alla loro germinazione.

Dalla germinazione di queste artrospore clamidosporioidi ha origine il micelio che potrà frammentarsi o meno in artrospore. Il micelio è composta da tratti miceliari ialini e da tratti miceliari opachi piú corti dei primi. Da entrambi i tratti miceliari, ma specie dai tratti miceliari opachi, hanno origine i rami laterali dai quali derivano catenelle a spore piú o meno seriate.

L'A. si sofferma a considerare l'interessante problema della funzione delle artrospore usuali non clamidosporioidi le quali non paiono essere sede di moltiplicazione, ma rappresentano lo stadio finale dell' evoluzione maturativa dei rami miceliari. Una volta sviluppatosi, il ramo micelico può essere sede di fissione o rimanere non scisso. In ogni caso tutti i rami miceliari sono potenzialmente sede di fissione, senza che possa individuarsi almeno morfologicamente in settori specializzati per la fissione o la continuità.

Il meccanismo di formazione delle artrospore iline è parso all'A. essere il seguente. Nell'ifa in fase che possiamo chiamare di pre-artrosporulazione, si delineano setti dati da zone trasversali irregolari di minor addensamento del plasma. Successivamente la zona chiara di separazione del plasma si delinea più nitidamente all'estremità per precipitazione di una sostanza rifrangente di probabile natura callosica, con interruzione della continuità protoplasmatica. In tal modo il protoplasma interstiziale e il residuo della

tunica miceliare continua primitiva, va rapidamente in lisi individualizzandosi così le artrospore in una catenella diritta o che segue le sinuosità del ramo miceliare che diede loro origine. L'ulteriore morfologia di queste artrospore varia probabilmente con fattori di ordine fisico-chimico riguardanti i processi osmotici che possono aver luogo tra il mezzo esterno ed il mezzo protoplasmatico e che si riflettono principalmente in modificazioni delle parete laterali delle artrospore, più sottile delle pareti basali, più spesse. Per tali fenomeni di osmosi le artrospore possono assumere l'aspetto a doppia mazza di tamburo, se isolate, a canna di bambù se seriate. Ció che si è detto riguarda le artrospore comuni che non abbiamo mai visto germinare e non quelle che abbiamo indicato come artrospore clamidosporioidi. Il fenomeno di irregolare distribuzione del contenuto plasmatico ha luogo anche nelle ife miceliche che non si divideranno per scissione. In questa il protoplasma e pure i nuclei migrano in parte, attraverso le cribrature dei setti, negli apici di crescita. Per tale fenomeno tratti miceliari possono svuotarsi di citoplasma ed apparire al microscopio come otticamente vuoti, in contrasto con l'opacità dei tratti miceliari densi di plasma e otticamente scuri.

Per quanto riguarda la classificazione sistematica dei ceppi studiati, l'A. discute il problema delle affinità di *Geotrichum* riferendo i Geotrichi da lui studiati al gruppo VII A (secondo Tubaki), cioè alla famiglia Geotrichaceae Cif. in senso stretto.

Per quanto si riferisce alla specie l'A. riporta tutti i ceppi studiati al *Geotrichum candidum* Link ex Persoon.

SUMMARY

Twelve strains of *Geotrichum* from human stool were studied from morpho-biological standpoint. The strains have been very homogeneous, especially on corn meal. The colony developed from short and stout arthrospores, here called chlamydosporoid arthrospores, having dense, opac plasma and double wall, particularly conspicuos germination. The mycelium is composed of light and dull segments; the side branches derive from both, chiefly from the duller segments. The lateral branches may produce usual, hyaline arthrospores by fission. The germination of the common arthrospore has never been observed, and its function in nature is unknown. The fission appears to be the end process of the hyphal differentiation. All the lateral branches are potentially capable of fission, apparently without previous morphological electivity of position.

The start of hyaline arthrospore formation is the appearence of pseudo-septa, made by transversal strand of denser plasma. The septum is better defined by a double, refractive line, apparently caused by precipitation of a refractive substance of probable callosic nature. The lysis of the interstitial plasma and of the segments of primitive hyphal wall, isolates the arthrospore. The final morphology of the mature, hyaline arthrospore is variable according to

certain physico-chemical environmental factors, above all to the osmotic processes, with some modification of the shape.

The irregular distribution of plasma content may be found even in not fissiparous hyphae, while the partial migration of plasma at the growing end of hyphae is a common phenomenon.

From taxonomic standpoint, the strains of *Geotrichum* are pertaining to the VII A group according to TUBAKI, namely, to the Geotrichaceae family according to CIFERRI.

All strains are referred to *Geotrichum candidum* LINK ex PERSON.

Letteratura citata

ARNAUD, G. & BARTHELET, J. (1936) — *Les microconidies dans le genre* Sclerotinia. Bull. Soc. Mycol. France, **52,** 63—79.

—, (1953) — *Mycologie concrète: genera II.* Bull. Soc. Myc. de France, **69,** 274.

BALDACCI, E., CIFERRI, R. & VACCARI, E. (1939) — *Revisione sistematica del genere* Malbranchea SACC. Atti Ist. Bot. Univ. Pavia, serie IV, **11,** 75—102.

BERKHOUT, C. M. (1923) — *De schimmelgeslachten* Monilia, Oidium, Oospora en Torula. Diss. Utrecht.

BOBROV, R. A. (1951) — *The nature and development of some strains of* Geotrichum. Unpublished thesis. University of California, Los Angeles (sec. CARMICHAEL).

BUBAK, F. (1912) — *Ein Beitrag zur Pilzflora von Sachsen.* Ann. Mycol. Notitiam Sci. Mycol. Univ., **10,** 52.

CARMICHAEL, J. W. (1954) — *A taxonomic study of the genus* Geotrichum. Unpublished thesis, Harvard University. (sec. *Carmichael*).

—, (1955) — *Lacto-fuchsin: a new medium for mounting fungi.* Mycologia, **47,** 611.

—, (1957) — Geotrichum candidum. Ibid., **49,** 820—830.

CIFERRI, R. (1958) — *Manuale di Micologia Medica.* Tomo I. Pavia.

—, (1958) — Mauginiella *a synonim* of Sporendonema. Atti Ist. Bot. Lab. Critt. Univ. Pavia, serie V, **15,** 126—133.

—, (1958) — *Sistematica, riconoscimento e frequenza delle* Candidae. Giorn. Malatt. Infett. Parassit., **1** (1).

—, & CHAVES BATISTA, A. (1956) — *A new genus of Phaeophragmious Dematiaceae with pseudoendogenous conidia.* Inst. de Micol., Publ. **44**. Recife.

—, VERONA, O. & SAGGESE, V. (1938) — *Reisolamento dello* Pseudomycoderma matalense *e revisione del gruppo.* Mycopathologia, **1** (3), 212—223.

—, (1930) — *Contribuzioni alla sistematica delle Torulopsidaceae.* Arch. Protistenk., **71,** 435.

— & REDAELLI, P. (1929) — *Studies on the Torulopsidaceae.* Ann. Mycol., **27,** 243—295.

COLONNELLO, F. (1944) — *Il* Geotrichum candidum *quale ospite dell'organismo umano.* Atti Ist. Bot. Lab. Critt. Univ. Pavia, serie V, **3,** 197—224.

DODGE, C. W. (1935) — *Medical mycology.* C. V. Mosby Co., St. Louis.

GOIDANICH, G. (1935) — *Miceti bolognesi.* Malpighia, **34** (1), 41.

HUGHES, S. J. (1953) — *Conidiophores, conidia and classification.* Canad. J. of Botany, **31,** 577—659.

LANGERON, M. (1945) — *Précis de mycologie.* Paris.

LINK, M. F. (1809) — *Observationes in ordines plantarum naturales.* Berlin. Magazin **3,** 3—42.

LODDER & KREGER VAN RIJ (1952) — *The Yeasts, a taxonomic study.* Amsterdam.

LOUBIÈRE, A. (1924) — *Recherches sur quelques Mucedinées caseicoles.* Thesis. Paris.

LUCHETTI, G. (1934) — *Comment se comporte le Geotrichum javanense Ver. dans le lait?* Boll. Sez. Ital. Soc. Intern. Microb., fasc. XII.

PERSOON, C. M. (1822) — *Mycologia europaea,* **1,** 26.

REDAELLI, P., CIFERRI, R. & DOMINICI, F. (1943) — *La microflora del cadavere umano.* Atti Ist. Bot. Lab. Critt. Univ. Pavia, serie V, **3** (2), 123—125.

—, — & — (1934) — *Studio comparativo di ventun ceppi di Sporendonema epizoum* (Corda) *nobis* (= *Hemispora stellata* Vuill.; *Oospora d'agatae* Sacc.). Ibid., serie IV, **5,** 145—198.

Robak, H. (1932) — *Investigations regarding fungi on Norwegian ground wood pulp and fungal infection at wood pulp mills.* Nytt. Mag. Naturvidenskab, **71,** 243—255.

Tubaki, K. (1958) — *Studies on the japonese Hyphomycetes.* V. *Leaf & Stem Group with a discussion of the classificatiom of Hyphomycetes and their perfect stages.* Journ. Hattori Bot. Lab., No. 20, 142—237.

Verona, O. (1933) — *Su di una bevanda tipo „yoghurt" dell'isola di Giava e sulle associazioni microbiche nella fermentazione acido-alcoolica del latte.* Boll. R. Ist. Sup. Agrario, Pisa, **9,** 475.

Windisch, S. (1951) — *Zur Biologie und Systematik des Milchschimmels und einiger ähnlicher Formen.* I. Beiträge Biologie Pflanzen, **28,** 69—130.

—, (1953) — Ibid. II. Milchschimmelähnliche Pilze, **29,** 143—170.

THE NUCLEAR MATERIAL IN STREPTOMYCES [1]

L. W. Durrell [2] & L. A. Schaal [3]

(27.IV.1959)

Whether the cells of *Streptomyces* contain nuclei or have nuclear material has been a question. As early as 1902 Neukirsk considered the staining of the fine granules in *Streptomyces* with methylene blue as evidence of the presence of nuclei. Krassilnikov considered these granules to be chromatin that acted as a nucleus. In 1930 and 1934, Jensen stained cells of *Microsmonospora* using Schumacher's method for nuclear material, which is similar to that of Feulgen. He was able to stain the minute granules in old spores and germinating cells. In 1934 Ripple obtained similar results with Feulgen technique but believed the stained bodies were fats. In 1940 von Plotho demonstrated the nuclei acid nature of these granules using the Feulgen method, illustrating the evidence with excellent photomicrographs. In 1947, Klienberger successfully applied to *Actinomyces* the nuclear method for staining bacteria. This method is similar to that of Feulgen but uses Giemsa stain. The work of Robinow, DeLamater, Bradfield and others on the nuclear material in bacteria, and the cytological studies Brachet and associates suggests better methods of identifying the granules in the cells of *Streptomyces*.

In reconsidering the question, the evidence that certain *Streptomyces* readily vary or mutate following exposure to ultra violet light of 2537—2600 Å, leads one to believe that the minute cells of the organisms contain D.N.A. that absorbs this region of light. The cells of *Streptomyces* are so small, 3μ or less, that it is hard to visualize an organized nucleus as we usually think of one. Stained granules can readily be observed and unstained ones can be seen with a phase contrast microscope. This however does not give evidence of their nature.

To focus evidence more precisely, aerial cells of a strain of *Streptomyces*, # 471 from potato tubers was used. Chiefly because the individual cells were comparatively large.

---

[1] Cooperative investigations of Colorado Agricultural Experiment Station and Horticultural Crops Research Branch. A. R. S. U.S. Department of Agriculture. Published with the approval of the Agriculture Director of the Colorado Agricultural Experiment Station as Scientific Series Paper No. 612.

[2] Botanist, Emeritus. Botany and Plant Pathology Section, Colorado Agricultural Experiment Station.

[3] Senior plant pathologist. Horticultural Crops Research Branch, Agricultural Research Services, U.S. Department of Agriculture.

To break up the clumps, masses of cells were shaken in 2 or 3cc. of sterile water to which a small crystal of sodium lauryl sulfate was added. In this strain of *Streptomyces* as in many others, the cells are covered with a fatty layer that interferes with the microscopic observation. It was found that the addition of a lipase solution to the cell suspension, 10mg approximately, removed the layer, judging from microscopical appearance. This solution was added from a sterile syringe through a Swinny filter to avoid contamination from bacteria that were in the enzyme powder. After treatment the walls of the adjoining cells touched, whereas before they appeared separated by a space the thickness of the coverings.

The suspension of cells was placed on slides with a fixative and held over acetic acid, allowed to dry on the slide, and then stained by the standard Feulgen technique. The result showed purple granules in the cells as referred to by others.

Methyl green a stain for D.N.A. was used and the same central granules were evident, though not as distinct as with the Feulgen method. Pyronin a stain for R.N.A. was tried on the cells, the effect was negative. Likewise Janus green, a stain for mitochondria, was used but did not stain the granules. Toludine blue was tested, this gives a red stain for metachromatic granules, common to bacteria and fungi, the granules however did not stain red.

In addition the *Streptomyces* cells were treated with ribonuclease, approximately 2 mg. per c.c and then stained by Feulgen, no difference was evident from the slides previously treated. Finally the cells were treated with Desoxy-ribonuclease followed by the Feulgen test. Here the cells were blank and no stained granules were evident. An exception to this was a few cells in the center of small clumps where the enzyme evidently did not have time to penetrate, these contained stained granules, indicating that the Feulgen reaction was functioning. Isolated cells and those at the margin of the clumps were clear and showed no granules.

These results point to D.N.A. as the constituant of the granules in the *Streptomyces* tested.

Pertinent Literature

Brachet, Jean (1953) The use of basic dyes and Ribonuclease for cytochemical detection of ribonucleic acid. J. micros. Soc. **94:** 1.

Brachet, Jean (1957) Biochemical Cytology. Acad. Press. Inc.

Bradfield, J. R. G. (1954) Electron microscopic observations on bacterial nuclei. Nature **173:** 184.

Chevremont, M. & Chevremont-Comhaire, S. (1955) Penetration of Ribonuclease in Vivo. C. R. Soc. Biol. **149:** 1525.

Danielli, J. F. (1958) General Cytochemical Methods. Acad. Press. Inc.

Delamater, E. D. (1951) A staining and dehydrating procedure for the handling of microorganisms. Stain Tech. **26:** 199.

— & Woodburn, M. (1952) Evidence for the occurrence of mitosis in Micrococcus. J. Bact. **64:** 797.

Jensen, H. L. (1930, 1932) The genus Micromonospora a little known group of soil organisms. Proc. Linn. Soc. N. S. W. **55:** 231—249, **57:** 175—180.

KLIENBERGER, N. E. (1947) The life cycle of sporing actinomyces as revealed by a study of their structure and septation. J. gen. Micro. biol. **1:** 22—32.

LIESKE, R. (1921) Morphologie und Biologie der Strahlenpilze. Leipzig, Borntraeger.

NECAS, O. (1958) Action of Ribonuclease on yeast protoplast. Exp. Cell Res. **14:** 216.

NEWCOMES, E. H. & KNIGHT, G. K. (1939) Nuclei in Actinomyces. Michigan Acad. Sci. Arts & Letters **25:** 85—87.

PLOTHO, O, VON (1940) Beiträge zur Kenntnis der Morphologie und Physiologie der Actinomyceten. Arch. Mikrob. **11:** 33—72.

—, (1940) Die chromatische Substanz bei Actinomyceten. Arch. Mikrob. **11:** 285—311.

RIPPLE, A. & WITTER, P. (1934) Untersuchungen an Actinomyceten. Arch. Mikrob. **5:** 24—30.

SAITO, H. & IKEDA, Y. (1957) Streptomyces griseoflavus a genetic study. Experientia **13:** 473.

EXPERIMENTAL HISTOPLASMOSIS IN COLD-BLOODED ANIMALS [1], [2])

GEORGE H. SCHERR & JOHN W. RIPPON [3])

Department of Microbiology, The University of Illinois College of Medicine, Chicago

(16.VI.1959)

The importance of 37° C incubation temperature and SH-containing compounds has been established for the mycelium (M) to yeast (Y) conversion of the dimorphic fungus *Histoplasma capsulatum* in vitro (SALVIN, 1949; SCHERR, 1957). The dimorphism phenomenon has been reviewed by SCHERR & WEAVER (1953). In order to determine whether this conversion would occur in vivo at temperatures other than 37° C and whether infection by the organism requires the yeast phase, as appears essential for the establishment of this disease in mammals, experimental histoplasmosis was produced in poikolothermic animals, using both the mycelium (M) and yeast (Y) phase of *H. capsulatum.*

Studies on experimental histoplasmosis in cold-blooded animals has been reported, to our knowledge, by two authors. MEYERS & SHERWOOD (1951) reported no infection when four frogs injected with a spore suspension were incubated at 33° C. Of four frogs (*Rana pipiens*) similarly injected and incubated at 25° C., one showed generalized systemic infection with the appearance of encapsulated yeasts on impression smears from liver and kidney. In a larger experiment, PINE & PEACOCK (1958) injected twenty-five toads (*Bufo marinus*) with Y or M phase of *H. capsulatum* and incubated them at 25° or 35° C. They observed no disease in M phase inoculated toads incubated at 25° C. In other frogs yeast cells were found in lesions at the site of inoculation but may have represented part of the original inoculum since no evidence of phagocytosis was presented.

---

[1]) The study reported here was supported by Grant No. E-1651 from the U.S. Department of Health, Education, and Welfare, the National Institutes of Health, and the University of Illinois Graduate Research Fund.

[2]) A portion of this report was presented at the 59th Annual Meeting, Society of American Bacteriologists. (RIPPON & SCHERR, 1959).

[3]) This report is taken from a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Illinois Professional Colleges.

Materials and Methods

Yeast cells of *H. capsulatum* were harvested with saline from seven day old brain-heart-infusion (BHI) blood agar slants. The cells were centrifuged at 1500 RPM for fifteen minutes, decanted, and resuspended in saline to a final concentration of 10^8 cells/ml. Mycelial-phase cells were serially transferred four times in glucose broth at 25° C to eliminate yeast cells. Ten-day tranfers of mycelial growth were decanted, suspended in saline, shaken with glass beads for thirty minutes on a rotary shaker at 140 RPM, and resuspended in saline at a concentration approximately equal in turbidity to 10^8 cells/ml yeast cells.

Frogs (*Rana pipiens*) and lizards (*Sceloporus undulatus* and *Eumeces fasciatus*) were inoculated intraperitoneally with yeast and mycelial phase suspensions (0.3 ml.) of *H. capsulatum.* The frogs were maintained in jars with a half inch of water, and the lizards were housed in cages with damp sphagnum moss. Groups of animals were incubated at 25°, 30° and 37° C and were autopsied at various times post injection. Cavity fluid and lesion material from organs were cultured on BHI blood agar slants at 37° C and on Sabouraud's glucose agar at 25° C. Impression and squash smears of organs and lesion material were stained by the Hotchkiss-MacManus method.

Results

Tables I and II summarize the autopsy findings on frogs injected with M or Y phase cells of *H. capsulatum* and incubated for sixteen days. The frogs were unable to survive incubation at 37° C and all died within 24 hours. At 25° and 30° C the lesions were primarily

Table I.

Experimental Histoplasmosis in Cold-blooded Animals

Frogs (six groups, two animals per group) injected I.P. with saline suspensions of *H. capsulatum,* mycelium and yeast phase. Animals incubated at 25 and 37 C. Autopsy 16 days post injection. Summary of findings.

(All animals incubated at 37 C. died within 24 hours.)

Autopsy Results	
Cultural phase injected	Incubation Temperature 25 C.
Yeast	a. Negative b. Lesion on liver *)
Mycelium	a. Lesion on liver *) b. Lesion on liver *)
Control (normal frogs)	a. Negative b. Negative

*) Hotchkiss-MacManus stained slide of lesion material showed only mycelium in tissue.

TABLE II.

Experimental Histoplasmosis in Cold-blooded Animals

Frogs (four groups, three animals per group) injected I.P. with saline suspensions of *H. capsulatum*, mycelium and yeast phase. Animals incubated at 25 and 30 C. Autopsy 12 days post injection. Summary of results.

Cultural Phase Injected	*Autopsy Results* Incubation Temperature 25 C.	 30 C.
Yeast	a. Lesions on liver *) b. Lesions on liver *) c. Lesions on liver *) organism recultured	a. Lesions on Liver *) b. Lesions on liver *) c. Lesions on liver *) organism recultured
Mycelium	a. Lesion on liver *) b. Lesion on liver *) c. Lesion on liver *) organism recultured	a. Lesion on liver *) b. Lesion on liver *) c. Lesion on liver *) organism recultured

*) Hotchkiss-MacManus stain of lesion material showed mycelium only in tissue.

mycelial, regardless of the growth phase injected. In both experiments the lesions were found only on the liver, from which the organisms could be recultured. Squash smears stained by the Hotchkiss-MacManus method showed extensive mycelial infiltration. The organism was not abundant in lesion material, though hyphal infiltration of tissue surrounding the lesion was extensive. The organism could not be recultured from organs other than the liver.

In the third series of experiments (Table IIIa, b) the organism was injected into frogs and studied at periodic intervals. Within twenty-four hours clusters adherent to the liver were noted in frogs

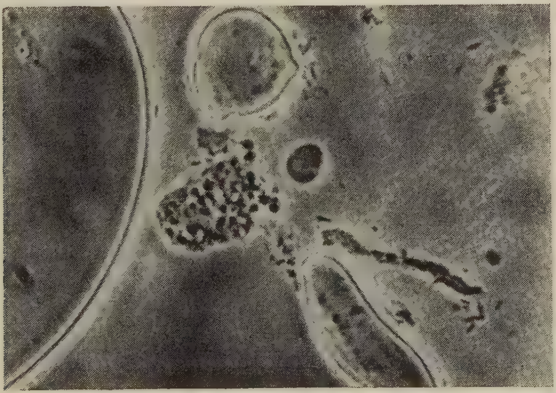


Fig. 1. *H. capsulatum* yeast phase in macrophage cells of frog. Wet mount. Phase microscope, 440x.

TABLE III a.

Frogs (eighteen groups, three animals per group) injected I.P. with saline suspensions of *H. capsulatum*, mycelium and yeast phase. Animals incubated at 25, 30 and 37 C. Autopsy at 24 hours, 48 hours and 15 days post injection. Summary of results.

(All animals incubated at 37 C died within 24 hours post injection.)

		Autopsy Results	
Cultural Phase Injected	Time Post Injection	Incubation 25 C.	Temperature 30 C.
Yeast	24 hrs.	a. Y cells phagocytized and in peritoneal fluid b. Y cells present in peritoneal fluid c. Y cells phagocytized and in fluid	a. Y cells present in peritoneal fluid b. Y cells phagocytized and free in fluid c. Y cells phagocytized and in fluid
	48 hrs.	a. Y cells present shows sign of conversion to M phase b. Y cells phagocytized and converting to M phase c. Y cells present in fluid and phagocytized	a. Y cells present in fluid and phagocytized b. Similar evidence of conversion to M phase c. Similar evidence of conversion to M phase, phagocytized and in fluid
	15 days	a. Lesion on liver extensive lesions on spleen and other organs *) b. Same *) c. Same *)	a. Died 3 days post injection. Mycelium in liver lesion *) b. Died 5 days — same* c. Died 9 days post injection. Lesions throughout cavity *)

*) Hotchkiss-MacManus stain of lesion material showed only mycelium in tissue.

injected with the M phase; stained smears showed these to consist solely of mycelial elements. Macrophage cells with phagocytized yeast cells were noted in the frogs injected with Y phase cells (Fig. 1) After forty eight hours frogs injected with Y phase cells showed the presence of short mycelial elements along with some phagocytosis of yeast cells by macrophage cells. Scattered nodules were noted after a similar time adherent on organs of frogs injected with the M phase. These nodules were solely mycelial in nature on stained smears. After two weeks lesions containing M phase cells exclusively were common in all these groups.

Lizards and frogs were inoculated in the fourth series of experiments. The frogs injected with Y phase cells incubated at 25° C

TABLE III b

Experimental Histoplasmosis in Cold-blooded animals

Frogs (eighteen groups, three animals per group) injected with saline suspensions of *H. capsulatum*, mycelium and yeast phase. Animals incubated at 25 and 30 C. Autopsy at 24 hours, 48 hours and 15 days post injection. Summary of results.

		Autopsy Results	
Cultural Phase Injected	Time Post Injection	Incubation Temperature	
		25 C.	30 C.
Mycelium	24 hrs.	a. Mycelium present	a. Adherent spots on liver. Direct smear-mycelial clusters.
		b. Same	b. Mycelium present
		c. Same	c. Same
	48 hrs.	a. Mycelium in peritoneal fluid, nodules on liver-mycelial clusters	a. Mycelium in peritoneal cavity, scattered nodules on omentum, liver, kidney
		b. Same	b. Nodules on liver-mycelial
		c. Same	c. Same
	15 days	a. Died 3 days post injection, mycelial lesions on liver *)	a. Died 3 days post injection, lesions on liver *)
		b. Died 5 days post injection, extensive lesions in liver	b. Died 3 days post injection — same *)
		c. Died 15 days post injection, numerous lesions throughout cavity, in liver, kidney. Extensive mycelial infiltration*	c. Died 3 days — same*

*) Hotchkiss-MacManus stain of lesion material showed only mycelium in tissue.

and autopsied at three weeks post injection showed histologic findings similar to those in previous experiments (Table IV). Lesions were extensive and mycelial in nature, with occasional evidence of phagocytized yeast. Three frogs injected with M phase cells succumbed by the sixteenth day. Extensive mycelial invasion of most internal organs was noted. The organism was recultured from liver, kidney and spleen. The two M phase injected frogs to survive three weeks showed scarring and adhesions on internal organs. *H. capsulatum* could not be recultured from these frogs.

Lizards (*Sceloporus undulatus*) injected with *H. capsulatum* died at varying times post injection. In the lizards (Table Va, b) incubated at 25° C for up to three weeks infection was noted as lesions on liver and spleen; these were mycelial regardless of cultural phase injected. Some yeast cells persisted in Y phase injected lizards

TABLE IV.

Experimental Histoplasmosis in Cold-blooded Animals

Frogs (four groups, five animals per group) injected with saline suspensions of *H. capsulatum*, mycelium and yeast phase. Animals incubated at 25 C. Autopsy 25 days post injection. Summary of results.

		Autopsy Results
Cultural Phase Injected	Time Post Injection	Incubation Temperature 25 C.
Yeast	25 days	a. Several lesions on liver, extensive mycelial invasion *), organism recultured b. Lesions on liver and peritoneum *), recultured c. Lesions on liver *), recultured d. Lesions on liver *), recultured e. Several large lesions in liver and kidney *), recultured
Mycelium	Died in 16 days	a. Extensive lesions on liver *)
	Died in 16 days	b. Extensive lesions on liver *)
	Died in 16 days	c. Lesions on liver *), recultured
	25 days	d. Scarring and adhesions-culture negative e. Adhesions-slide and culture negative

*) Hotchkiss-MacManus stain of lesion material showed only mycelium in tissue.

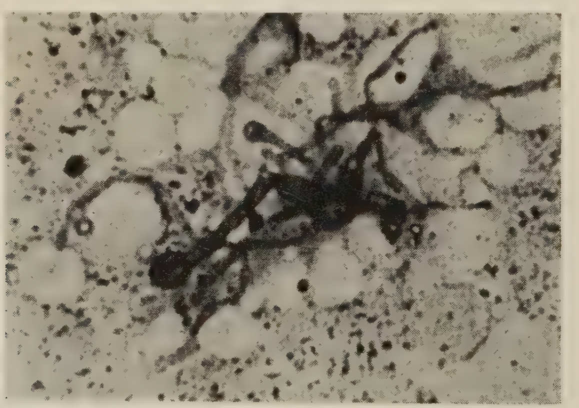


Fig. 2. *H. capsulatum* in liver smear from lizard showing mycelium and chlamydospore. Hotchkiss-MacManus stain, 440x.

throughout the incubation period. Lizards injected with Y phase and maintained at 37° C showed phagocytized and free yeast cells in lesion material for periods up to three weeks. The organism was

TABLE V a.

Experimental Histoplasmosis in Cold-blooded Animals

Lizards (*Sceloporus undulatus*, four groups, five animals per group) injected with saline suspensions of *H. capsulatum*, mycelium and yeast phase. Animals incubated at 25 and 37 C. Autopsy at death or by 22 days. Summary of results.

Cultural Phase Injected	Time Post Injection	Incubation Temperature	Autopsy Results
Yeast	Died in 2 days	25 C.	a. Yeast recovered from peritoneum b. Same with evidence of pseudomycelium
	Died in 4 days		a. Yeast in peritoneum, spot on liver, pseudomycelium
	Died in 8 days		a. Yeast in macrophage, mycelium present
	Died in 13 days		a. Yeast in macrophage, large lesion on liver containing mycelium *)
Yeast	Died in 1 day	37 C.	a. Yeast in peritoneum b. Same
	Died in 4 days		a. Yeast in macrophage
	Died in 9 days		a. Liver lesion present containing yeast cells on direct smear and stain *)
	22 days		a. Several liver lesions present containing yeast cells on direct smear and stain *)

*) Organism recultured

recultured from lesion material of internal organs. Conversion to yeast phase occurred in lizards injected with M phase and incubated at 37° C; the lesion material showed mycelial elements along with free and phagocytized yeast cells.

The experiment was repeated using another species of lizard (*Eumeces fasciates*) with essentially the same results.

DISCUSSION

The dependence upon environmental temperature for the conversion of the dimorphic fungus *Histoplasma capsulatum* to yeast phase growth was demonstrated by growth in cold-blooded animals. The lesions produced in the animals incubated at 25° or 30° C ultimately became solely mycelial in nature, regardless of the growth phase injected. If similar animals were injected with M phase and incubated at 37° C, infection resulted with conversion to the Y phase. Animals injected with Y phase and similarly incubated showed yeast cells in lesion material at either temperature.

TABLE V b.

Experimental Histoplasmosis in Cold-blooded Animals

Lizards (*Sceloporus undulatus*, four groups, five animals per group) injected with saline suspensions of *H. capsulatum*, mycelium and yeast phase. Animals incubated at 25 and 37 C. Autopsy at death or by 22 days. Summary of results.

Cultural Phase Injected	Time Post Injection	Incubation Temperature	Autopsy Results
Mycelium	Died in 3 days	25 C.	a. Mycelium present in peritoneum b. Same
	Died in 9 days		a. Liver lesions containing mycelium by stained smear *)
	Died in 13 days		a. Lesion on liver-mycelium *)
	22 days		a. Lesions in liver, wet mount and stain-mycelium with chlamydospores present *) (Fig. 2)
Mycelium	Died in 1 day	37 C.	a. Mycelium present in peritoneum
	Died in 6 days		a. Mycelium present in peritoneum
	Died in 9 days		a. Mycelium and occasional yeast present in liver lesion by direct smear and stain *)
	Died in 13 days		a. Yeast in macrophage and as free cells, some mycelium present *) b. Same *)

*) Organism recultured

This observation is consistent with the in vitro results showing that Y phase cells of *H. capsulatum* could under certain conditions be maintained at 25° C for weeks (SCHERR, 1957).

The experimental evidence presented here strongly implies that the metabolic pathway permitting, and/or the process directing, conversion to the yeast phase is inherent in the fungus itself. This pathway has not yet been shown to become active at a temperature less than 37° C. At 25° C disseminated infection with mycelial phase cells of *H. capsulatum* can occur in cold-blooded animals. In mammals this does not appear possible, for the environment of the host is incompatible with M phase multiplication (see SCHERR, 1957).

If 37° C permits the functioning of a pathway for M→Y conversion of this fungus (and other related dimorphic organisms), then one might be able to isolate and identify essential metabolic intermediates active in this pathway at 37° C. By introducing such metabolites into the M phase culture growing at 25° C, one might satisfy this specific deficiency enough to effect the M→Y conversion. Such experiments are presently underway.

Bibliography

MYERS, W. F. & SHERWOOD, N. P. (1951) Experimental histoplasmosis in the grass frog Rana pipiens. Bact. Proc. 114.

PINE, L. & PEACOCK, C. L. (1958). Studies on the growth of *Histoplasma capsulatum.* IV. Factors influencing conversion of the mycelial phase to the yeast phase. J. Bact., **75:** 167—174.

RIPPON, J. W. & SCHERR, G. H. (1959) Experimental histoplasmosis in cold-blooded animals. Bact. Proc. **82.**

SALVIN, S. B. (1949) Cysteine and related compounds in the growth of the yeastlike phase of *Histoplasma capsulatum.* J. Inf. Dis., **84:** 275—283.

SCHERR, G. H. (1957) Studies on the dimorphism of *Histoplasma capsulatum.* I. The roles of -SH groups and incubation temperature. Exp. Cell Res. **12:** 92—107

SCHERR, G. H. & WEAVER, R. H. (1953) The dimorphism phenomenon in yeasts. Bact. Rev., **17:** 51—92.

OBITUARY

Giovanni Battista Traverso

Il prof. G. B. Traverso, deceduto il 22 di gennaio 1959, era nato a Pavia il 25 ottobre 1878.

A Pavia Egli aveva compiuto i suoi studi, laureandosi in scienze naturali nel luglio 1900 con il suo primo maestro Giovanni Briosi, ordinario di botanica e direttore del Laboratorio crittogamico italiano, annesso a quella cattedra. Già negli anni universitari, il Traverso aveva avuto modo di pubblicare in quella serena atmosfera di lavoro che il Briosi aveva creato nel suo Istituto, alcuni lavori su argomenti di botanica e di micologia.

Dopo la laurea, il Nostro passò per qualche breve tempo a Modena presso quella Stazione sperimentale agraria e poi a Padova con il Saccardo che teneva cattedra di botanica. Nel 1905 otteneva la libera docenza in botanica generale e fino al 1914 restava assistente in quell'Istituto, dove dava inizio ai lavori micologici di più ampia mole e si formava a quella Scuola micologica che onora l'Italia in tutto il mondo. Nel 1915 passava per concorso alla Stazione di patologia vegetale di Roma, come vicedirettore, dove aveva la fortuna di trovare un altro grande Maestro, Giuseppe Cuboni, che dedicava la sua alta cultura e il suo profondo pensiero ad aprire alla ricerca fitopatologica nuove vie.

Di questo periodo sono i maggiori lavori del Nostro su argomenti di patologia vegetale, fra i quali ricordiamo gli studi sulla peronospora del frumento e sulla malattia del castagno, detta "mal dell' inchiostro"; alcune malattie di origine batteriche, nonchè notevoli lavori di micologia.

Con il novembre 1923 è professore al R. Istituto superiore di Agricoltura di Milano, poi trasformato in Facoltà di Scienze Agrarie. A Milano resto affezionato per tutta la sua vita; lasciò la cattedra nel novembre del 1948, ma continuò a dare la Sua opera come preside della Facoltà e poi come professore emerito.

Il Suo lavoro fondamentale dà inizio alla Flora Italica Cryptogamica, raccolta di volumi dedicati alla descrizione e alla diagnosi dei funghi di cui egli compilò i primi quattro; il primo dedicato alla Bibliografia della Micologia Italiana e i successivi al gruppo dei Pirenomiceti, dei quali fu uno dei maggiori specialisti di tutto il mondo. Con la scomparsa del Saccardo è fra i continuatori della sua opera e sono Sua fatica i volumi XIX e XX della grande Sylloge Fungorum che costituisce una opera colossale unica al mondo e recentemente ristampata in U.S.A.

Nella stessa opera troviamo la Sua collaborazione ai volumi XVII,

Giovanni Battista Traverso
25.10. 1878—22 1. 1959

XXIII, XXIV. Non dimenticò mai i Suoi Maestri; se di SACCARDO continuò l'opera, di CUBONI raccolse con amore di discepolo gli scritti più originali e più profondi di insegnamento, in un volume che ancor oggi si legge con gran diletto e qui ne tracciò la biografia. Di un altro micologo, del BRESADOLA di Trento, Egli ne promosse pure le onoranze e ne curò con grande intelligenza la pubblicazione postuma della opera dedicata sopratutto ai Basidiomicetti.

Scompare così con TRAVERSO un altro grande studioso italiano che ha onorato la Patria nelle scienze ed in particolare nel campo della micologia e della patologia vegetale, nel quale gli Italiani ognora sono stati fra i primi.

ELIO BALDACCI

LIBRI NOVI

Ciferri, Rafaele and Piero Redaelli: *Bibliographia Mycopathologica* (1800—1940). Vol. I. Authors A—K. Titles 1—7260 pp. 408. Vol. II. Authors L—Z. Titles 7261—14506. pp. 399. Sansoni Antiquariato, Firenze (Florence), Italy. 1958.

Sansoni Antiquariato publishes two bibliographic series: Biblioteca Bibliografica Italica, and Contributi alla "Biblioteca Bibliografica Italica". These two volumes constitute Volumes 18 and 20 in the second of these series.

A brief introduction states that for more than 30 years a card file of the literature of mycology dealing with the diseases of man and animals has been developed in the Center of Medical Mycology, University of Pavia, Italy. Titles in this file number more than 14,500 for the 140 year period covered by this bibliography. A quick survey of subject matter indicates that most titles deal with fungus diseases of man.

References to animal diseases and diseases of insects are included, as are items on predacious fungi associated with nematodes, amoebae, and other animals. Some titles referring to allergy problems related to fungi are included.

References to topics related to these subjects, including general texts, are listed. All titles are listed alphabetically by author.

Most titles are given in English, French, German, Italian, Spanish or Portuguese. If titles have been transliterated from languages using non-Roman type, this has not been indicated. If such titles are not included in this listing, it is probably because of reasons cited in the introduction to Vol. I.

References are printed clearly on fairly heavy paper. The paperback binding seen by this reviewer seems to be serviceable. References are numbered consecutively. They include name of author, year of publication, title, name of journal abbreviated, volume number and pagination. The system of abbreviating journal names is not cited but usually these names are not unnecessarily foreshortened.

Only 666 copies of this useful bibliographic contribution were printed. It should be available to all medical and veterinary mycologists.

Wm. Bridge Cooke

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

SEGRETAIN, G., DROUHET, E., MARIAT, F.: Diagnostic de Laboratoire en Mycologie Médicale. Service de Mycologie de l'Institut Pasteur. Collection "Techniques de Base". Editions de la Tourelle, 5, rue Guynemer, Saint Mandé (Seine). Paperback, 16⁰, 143 pages, 1958.

The noted triumvirate of the Mycologic Service at the Pasteur Institut produced a very handy and most useful laboratory guide. In seven chapters they covered the whole field of the mycologic technique. There is a short, succinct introduction into the general morphology and physiology of fungi. The collection of material, inoculation of nutrient media, identification of species and determination of the pathogenicity of the microorganisms are well covered. Further chapters deal with mycotic affections of the mucous membranes, the superficial and deep mycoses. A useful formulary of the nutrient media and staining agents are added. There is also a glossary of the basic mycologic terms. The more important texts on mycology are quoted. Twenty-five drawings illustrate the guide depicting the basic features of the commonest fungi, in adding further usefulness to the guide. It is clearly written, considering all the necessary details in short, concise presentation. Paper, print are satisfactory. This guide can be warmly recommended for general laboratory use not only for the beginner but also for the advanced student in mycology for a quick reference.

TIBOR BENEDEK

Diseases of Metabolism. Detailed methods of diagnosis and treatment. GARFIELD G. DUNCAN, editor. 1104 pages. W. B. Saunders Co., Philadelphia and London, 1959.

The fourth edition of this popular textbook is published seven years after its predecessor. The staff of contributors has been changed extensively, although the material covered is subdivided as in the past. Eleven of the 22 authors are collaborating on this volume for the first time. The format has been changed to a two column lay-out, which improves the ease of reading. The author index has been deleted. A remarkable job of editing has been done: despite the very considerable additions to our knowledge in the broad field of human metabolism which has accumulated in recent years, Dr. DUNCAN has been able to keep this volume at the length of the previous edition.

The 18 chapters cover normal metabolism and its derangements adequately and in lucid fashion. The approach is primarily clinical, although fundamentals of the metabolic pathways are also described with unusual clarity. Therapeutic recommendations largely are interwoven with the physiologic exposition and the rationale of treatment is emphasized. Throughout the book commendable use is made of diagrams and tables to both shorten and elucidate the text. Each chapter is followed by an extensive bibliography.

"Diseases of Metabolism" can be recommended as an excellent, up to date text for the medical student, general physician and internist.

THOMAS G. BENEDEK

Department of Medicine
Veterans Administration Hospital
Pittsburgh, Pa.

Modern Dermatologic Therapy. Edited by THOMAS H. STERNBERG, M.D. and VICTOR D. NEWCOMER, M. D. 520 XII pp. McGraw-Hill Book Company, Inc. 1959.

This book consist of reviews of twenty-six largely unrelated subjects by twenty-two contributing authors under the able editorial guidance of Doctors STERNBERG and NEWCOMER. The range of subject matter is very broad and includes such diverse topics as the collagen diseases, psychiatric aspects of dermatology, fungous infections and the treatment of syphilis with antibiotics other than penicillin. Naturally, the quality of the individual chapters is far from uniform although the editors have evidently done their part well.

A few of the chapters are superbly done while others offer little which is not more succinctly available in modern texts. The authoritative discussion of Miliaria with excellent photographic and diagrammatic illustrations and the chapter on pigmentary disorders are excellent. In the reviewer's opinion, they defy criticism.

The lead article on the symptomatic management of lupus erythematosus stresses the use of steroid therapy. This group of treatment agents is repeatedly mentioned in many other discussions throughout the book, testifying to its usefulness in dermatology.

There is little in this book which will be of primary interest to the mycologist and this is especially true of the chapter on superficial mycotic infections. It is assumed that the statement on page 410 suggesting that three negative cultures be obtained at 27 week intervals, before pronouncing as cured tinea capitis due to M. *canis* or *M. audouini*, is a typographical error. Recent advances in the therapy of superficial fungous infections have greatly reduced the value of the material presented in this contribution.

The bibliographies at the end of each chapter appear to have been judiciously selected. Although the accuracy of the references has not been systematically tested it is probable that this element in the book constitutes one of its strongest features.

IRENE NEUHAUSER

University of Illinois, College of Medicine, Chicago, Ill.

The Biology of Hair Growth, edited by W. MONTAGNA and R. A. ELLIS, Academic Press, Inc. New York and London, 1959. Price: $ 15.—.

This comprehensive volume is an outgrowth of a conference on The Biology of Hair Growth, held at the Royal College of Surgeons, in London, on August 7,8 and 9, 1957. The papers presented at this conference, and a few others, have been gathered in this book to serve as a source reference for all those interested in research on hair and hair growth. Twenty-three authors contributed to this volume, every one of them an authority in his own chosen field. Morphology, biology and biochemistry of the hair and its growth are thoroughly discussed. The highly important field of pigment, its formation and its loss are well covered. Age, sex and genetic factors are sufficiently considered. MONTAGNA, besides his own contribution, along with ELLIS did an outstanding editorial job. Illustrations are well chosen and satisfactory. Every chapter has its own extensive bibliography. Author and subject indices are added. Paper, printing and binding are outstanding. This work is highly recommended to all those who are interested in "basic" science research, among them to medical mycologists on account of the practically important and biologically utterly complicated processes of fungus infections of the scalp, from the standpoint both of the infective as well as of the healing process.

TIBOR BENEDEK

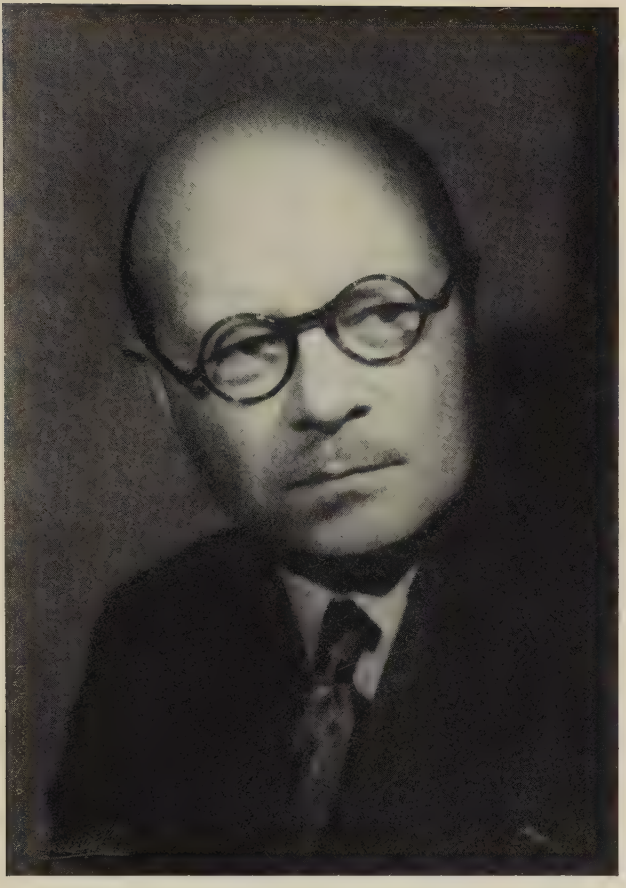
ANNOUNCEMENT

Attention is called to a bibliography on FUNGUS INFECTIONS, covering the literature of 1952 through September 1958, compiled by DOROTHY BOCKER, M. D. Medical Officer, Public Health Service, U.S. Department of Health, Education, and Welfare. This bibliography is designed as a tool for clinicians and laboratory workers. It is not exhaustive, but attemps to present a selection of the literature. References are in English, French, German, Italian or Spanish, occasionally annotated. The references are arranged in three groups: (1) General, (2) Systemic infection, (3) Superficial infection. It contains 942 references. It is available without charge through the U. S. Public Health Service, Washington 25, D.C.

E. BALDACCI

Istituto di Patologia Vegetale
Università di Milano, Italia.

Gallery of Contemporary Noted Mycologists
53



R. HEIM

Directeur du Muséum National d'Histoire Naturelle de Paris

Membre de l'Académie des Sciences.

Gallery of Contemporary Noted Mycologists

54

A. NANNIZZI

Istituto Botanico

Università di Siena, Italia.

Gallery of Contemporary Noted Mycologists

55

E. GÄUMAMN

Eidg. Technische Hochschule Zürich, Schweiz

Gallery of Contemporary Noted Mycologists

56

MOUSE FAVUS DUE TO TRICHOPHYTON QUINCKEANUM (ZOPF) MACLEOD & MUENDE: A REAPPRAISAL IN THE LIGHT OF RECENT INVESTIGATIONS

by

Charles J. La Touche, L. A. H. (Dubl.), M. Sc., M. I. Biol.
From the Department of Dermatology, the General Infirmary at Leeds, Yorks, England and the Department of Bacteriology, University of Leeds.

(15.V.1959)

> ,,L'un et l'autre nous cherchons la vérité pour elle, non pour avoir raison, ni pour faire du bruit. Entre nos arguments inverses elle se trouve cernée. L'avenir dira qui s'est le plus approché d'elle."
>
> R. Sabouraud

Part 1.

Introduction.

Review of literature.

Accounts of Favus in mice, cats and dogs and its transmission to Man by these animals, date back to the middle of the nineteenth century. It seems that Bennett (1) was the first to record the occurrence of favus in a mouse, although he did not describe it but contented himself with remarking merely that: "I have seen it on the face of a common house mouse, in which animal the same cryptogamic vegetations were to be detected as in Man. " This information appeared as a foot-note to a published lecture on favus in Man. Vanbreuseghem (2) quoted an excellent description of favus in mice by two Belgian observers, Gluge & d'Udekem (3). This appears to have been the first published account of favus in mice, although Bazin (4) and Saint-Cyr (5) cited a description of this disease in mice by Draper of New York which was communicated to them privately in 1854. The mice concerned had been given to a cat which, on becoming likewise affected, transmitted the infection to some children, and they eventually developed lesions with scutula on the glabrous skin. Amongst other early descriptions was that of FRIEDREICH (6) of a favic crust on the head of a mouse; and SAINT-CYR (l. c.) described spontaneous and experimental favus in cats, dogs and Man. Unfortunately, the observations of these and other authors of that period and during many years after, are, in regard to this subject, vitiated by their lack of cultural data; for although it is probable that most of these cases of favus in animals and Man were due to the organism which later became known most widely as *Achorion Quinckeanum* (ZOPF) BODIN, the existence of other etiologic agents of favus in Man and animals postulates possible alternatives, amongst which, one especially, *Trichophyton schönleini* (LEBERT) LANGERON & MILOŠEVIĆ, syn. *Achorion schönleini* (LEBERT) REMAK, was the most likely, because in those days, it was widespread as a cause of favus in Man, and is known to be transmissible to mice; in fact, it was considered to be the only etiologic agent of favus in both Man and animals until QUINCKE (7) claimed that he had isolated several etiologic agents of favus. These he labelled α-, β- and γ- Pilz.

QUINCKE'S claims for the multiple etiology of favus were much disputed by many of his contemporaries at the time that his work was published and for many years afterwards, because there were those who would not concede the possibility that favus could be caused by any other fungus than that which was already known to them, and those who, although they did concede this possibility, were unable to find evidence in support of it. SABOURAUD (8) gave an admirable account of this controversy in his famous classic "Les Teignes". It is sufficient therefore to add merely that discussions on this subject dragged on until the closing years of the last century. The question finally resolved itself during the early years of the present century when BODIN (9) discovered *Microsporum gypseum* (BODIN) GUIART & GRIGORAKI (syn. *Achorion gypseum* BODIN) in a scutulum-bearing lesion on the face of a woman aged 30 years, and BLOCH (10) showed that *Trichophyton violaceum* BODIN was capable of producing scutula in human lesions. Favus due to *Trichophyton gallinae* (MÉGNIN) SILVA & BENHAM [syn. *Achorion gallinae* (MÉGNIN) SABOURAUD] had already been known to occur in poultry, and SABOURAUD, SUIS & SUFFRAN (11) observed scutula in an experimental lesion which they induced in a human with this fungus.

Two other points, also connected with QUINCKE'S discovery, remained unresolved, and are still the subject of controversy, namely the authenticity of QUINCKE'S α - Pilz and its identity. QUINCKE neither classified it nor gave it a name, but nevertheless described clearly the features which it exhibited in culture. Further comment on his observations is relegated to the discussion at the end of this paper. (Part 4).

BODIN (12) who studied the etiologic agent of mouse favus in isolates both from mice and humans seemed to be in no doubt as to the similarity between them and QUINCKE's α - Pilz. As his conclusions were subsequently disputed by SABOURAUD (l. c.) it is necessary as well as of interest to quote here BODIN's own words regarding this matter. Thus ,he wrote: "d'abord il est évident que la mucédinée que j'ai rencontrée chez la souris et chez l'Homme et dont je m'occupe dans ce mémoire, offre les mêmes caractères que le champignon de QUINCKE, tant dans son aspect objectif en cultures que dans les lesions expérimentales ou non qu'elle produit chez l'Homme ou chez l'animal et qu'il s'agit bien en ce cas du parasite du favus de la Souris, ce terme étant pris dans le sens stricte que j'indiquais tout à l'heure." He thus seemed to be quite certain that his isolates were similar to QUINCKE's α - Pilz although his description of their microscopic features fell short of QUINCKE's in so far as it did not include any macroconidia. This, however, does not invalidate his conclusions since it is well known that the production of macroconidia in a number of dermatophytes is often erratic, and, in some cases, necessitates the presence of certain nutrient substances in the culture medium. SABOURAUD's (l.c.) main objection to the authenticity of QUINCKE's α — Pilz seemed to be that QUINCKE isolated it from a scutulum on the arm of a patient who was already suffering from human-type favus due to *T. schönleini*. In fact, QUINCKE, (l.c.) first isolated and first described it from a single lesion on the right side of a man's neck, and this patient was not suffering from human-type favus. It is nevertheless true that he subsequently (35) claimed to have isolated his α — Pilz again; this time from several members of a family already affected, to be sure, with human-type favus, and one can only conclude from this that SABOURAUD's reiterated objection (8) (13) (14) was directed towards this later publication by QUINCKE. In his opinion, favus due to two different dermatophytes could not occur concurrently on the same patients. Thus, (14) he wrote: "Or, l'expérience nous montre que de toutes les lésions faviques d'un malade, c'est toujours le même parasite qu'on isole et qu'un même malade n'abrite jamais deux favus différents." He also considered that QUINCKE's α — Pilz was a contaminant: "une moisissure d'impureté." It is curious that in his various allusions to this matter he never referred to the patient concerned in QUINCKE's earlier work (7).

That SABOURAUD's views did not reflect the general opinion is clear from the following statement by TOMASCEWSKI (15): "Seit ihrer Einführung durch SABOURAUD ist denn auch der QUINCKEische α — Pilz in einer allerdings kleinen Anzahl von Favus-fällen festgestellt worden: von BODIN, PLAUT, WANDEL, ADAMSON, BLOCH, CHAJES und mir; und alle diese Autoren haben die ursprünglichen Angaben von QUINCKE als im Wesentlichen vollkommen richtig bestätigt." And this is what BLOCH (16) himself said regarding this matter: "Nach dem Studium der aus den vier neuen Fällen gewonnenen Kulturen kann ich nicht den geringsten Zweifel mehr hegen, dass es sich stets um denselben wenig variablen Pilzstamm gehandelt hat, um den von QUINCKE zuerst beschriebenen Erreger des Mäusefavus, das *Achorion Quinckeanum.*"

During all these years the taxonomic status of "*Achorion quinckeanum*" was never satisfactorily determined, and is still *sub judice* to-day. As already mentioned, QUINCKE did not classify or name this fungus. ZOPF (17) called it *Oidium Quinckeanum* ZOPF, and BLANCHARD (18) seems to have been responsible for the combination *Achorion Quinckeanum* ZOPF. Later, BODIN (12) also used it and was subsequently wrongly indicated as the authority for it by the majority of authors. BODIN (9) considered that this dermatophyte had affinities with species belonging to the genera *Trichophyton* and *Microsporum.* MEWBORN (24) who, from his description of it,seems to have isolated it from a scutulum-bearing lesion of the scrotum as well as from a concurrent, adjacently situated lesion on the thigh without scutula, did not give it a name but considered that it was a species of *Trichophyton*: "megalosporon ectothrix of probable animal origin."

Evidently, TOMASCEWSKI (l.c.) formed the same opinion of MEWBORN'S isolate: "Ferner erhielt wohl MEWBORN von einem Fall mit Skutulis und einem Trichophytieherd am Bein eine Kultur des α — Pilzes." It would seem that SABOURAUD (8) accepted the binomial *Achorion Quinckeanum* ZOPF from BODIN because it coincided with his own concept of the genus *Achorion.* Also, as stated before, denying to QUINCKE the credit of having been the first to describe this dermatophyte, he transferred it to BODIN, placing his name after the binomial as the authority for it. BLOCH (l.c.) was in no doubt as to the affinities of this fungus with the *Trichophyton* group; thus on, p. 457, he wrote: "Wenn man lediglich die kulturellen Eigenschaften des *Achorion Quinckeanum* ins Auge fasst, sein üppiges Wachstum bei Zimmertemperatur, seine Vorliebe für kohlehydrathaltige Nährböden, sein ganzes morphologisches und biologisches Verhalten auf künstlichen Nährböden, so müsste man unfehlbar zum Schlusse kommen, dass wir es mit einem *Trichophyton* zu tun hätten." He also collected further evidence for this view by recording his observation of chains of spores in a lanugo hair. When OTA & LANGERON (19) introduced their new botanical classification of the Dermatophytes, they instituted three new genera, namely: *Sabouraudites*, *Grubyella* and *Bodinia,* while retaining the genera *Trichophyton* and *Endodermophyton,* but eliminating the genera *Microsporum* and *Achorion.* In redistributing the various species of dermatophytes amongst these genera they allocated *A. quinckeanum* in the genus *Sabouraudites* and thus, according to them, it became *Sabouraudites quinckeanus* (ZOPF) OTA & LANGERON. Later, this was confirmed by LANGERON & MILOŠEVIĆ (20). In the meantime, GUIART & GRIGORAKI (21) proposed their botanical classification in which they retained the genus *Microsporum* but redefined it as follows: "Nous faisons rentrer dans le genre *Microsporum* toutes les espèces donnant dans les cultures mères un

grand nombre de fuseaux; les cultures sont toujours poudreuses et duveteuses; le type du genre est le *Microsporum lanosum.* Ainsi compris, le nouveau genre *Microsporum* est beaucoup plus extensif que l'ancien: outre les agents des microspories humaines et animales, il comprend en effet les agents des trichophyties ectothrix microides du type *gypseum* de SABOURAUD, l'ancien *Achorion gypseum* et enfin l' *Epidermophyton inguinale* de l'eczéma marginé de Hèbra''. According to them *Sabouraudites quinckeanus* (ZOPF) OTA & LANGERON became *Microsporum quinckeanum* (ZOPF) GUIART & GRIGORAKI. However, in spite of these taxonomic reshufflings this dermatophyte continued to be known as *Achorion quinckeanum* in subsequent years, at least by the majority of those who were sufficiently interested in it to write about it. In recent years, VANBREUSEGHEM (l.c.) attempted to reassess its taxonomic status with only a few of its morphological features to serve his purpose. He concluded from his own data and those which he found in the literature that this dermatophyte was a species of *Trichophyton* sensu LANGERON & MILOŠEVIĆ (21). Still more recently, BLANK (7) reopened this question, and after what would seem to have been an exhaustive study of the cultural features of this dermatophyte, comprising many strains from both human and animal subjects, came to the conclusion that it was a species of *Microsporum* and that *M. quinckeanum* (ZOPF) GUIART & GRIGORAKI should be its proper binomial. His main reasons for reaching this conclusion were stated by him as follows:

1. The great majority of the macroconidia are pedicellate, and spindle-shaped with pointed tips . . . . .
2. The microconidia are elongate and similar to those seen in other *Microsporum* species.
3. The primary cultures grow quickly, and in the beginning are as flat as those of other *Microsporum* species.
4. . . . The fact that both *M. quinckeanum* and *M. gypseum* have never been found to affect hairs in cases of spontaneous infections may also be mentioned in this connection.

The American authors, CONANT et al. (22) unceremoniously included it in the group species *Trichophyton mentagrophytes.*

In the latest edition of its memorandum on the "Nomenclature of Fungi pathogenic to Man and Animals" (23), the Medical Mycology subcommittee of the British medical Research Council recommended that this dermatophyte should be referred to by the binomial *Trichophyton quinckeanum* (ZOPF) MACLEOD & MUENDE. Why "MACLEOD and MUENDE"? These authors (24) merely pointed out that the microconidia and macroconidia of this dermatophyte were of *Trichophyton* type; they did not actually use the aforesaid binomial. The first to do this seems to have been SKINNER et al. (25); their statement runs as follows "Colonies white, downy, Conidia and macroconidia formed: *Trichophyton quinckeanum.*" According to the rules of botanical nomenclature it would seem that the names of these authors should follow the binomial for which they were responsible. It should thus read: *Trichophyton quinckeanum* (ZOPF) SKINNER, EMMONS & TSUCHYA.

The foregoing review of the literature concerning this dermatophyte, while not exhaustive, is at least an attempt to reveal some of the more important observations and conclusions which have resulted from past efforts to elucidate its morphology and biology.

Original Observations.

The purpose of the investigations which are described in the pages which follow was to provide a basis for the reappraisal of the various problems associated with this dermatophyte.

To be consistent and to avoid misunderstanding, the binomial *Trichophyton quinckeanum*, or more briefly, *T. quinckeanum* will be used in the ensuing text.

Clinical features.

In this Department, during a period of about eight years (1951—1958 incl.) a total of 22 patients presented with lesions which were ascribed to infection by *T. quinckeanum.* Cultural evidence of this was obtained in the case of 16 of these patients. Of the remaining 6, from whom no cultures were obtained, 2 were microscopically positive, 3 were microscopically negative, and 1, a female child aged 4 yrs., could not be examined owing to lack of cooperation. As, however, these patients were familial contacts of patients from whom the dermatophyte was isolated they were included in the total number recorded as being infected by *T. quinckeanum.*

The main clinical features of the lesions presented by these patients, as well as their age and sex and the reference number of the strain of *T. quinckeanum* isolated from them, are set out in Table I.

The majority of the patients presented with lesions on the glabrous skin only. Scalp lesions were observed in only 3. The lesions on the glabrous skin were circinate, either uniformly erythematous or annular, and circular or elliptical (Plate I, fig. 2). The amount of desquamation, vesiculation, pustulation and crusting was variable; but most of them showed some degree of inflammatory response.

Definitely formed scutula were observed in the solitary lesions of only 2 of the patients. In one of these (M.M.) they measured from 1—2 mm. in diam. (Plate I, fig. 1); in the other (I.B.) they were still smaller and could be discerned only with a 5 × hand lens. They were yellowish, circumscribed, crateriform and raised slightly above the level of the surrounding skin. In the lesion presented

TABLE I.

Initials of Patient	Sex	Age	Strain No. of isolate	Number and sites of lesions					
				Scalp	Face	Neck	Arm	Leg	Trunk
F.T.	male	5 yr.	1	—	—	—	—	1	—
J.H.	male	14 yr.	2	—	1	—	—	—	—
K.N.	male	7 yr.	3	1	—	1	—	—	—
R.N.	female	13 yr.	—	—	—	—	—	—	1
P.N.	female	8 yr.	—	—	—	—	1	—	—
P.N.	male	5 yr.	—	—	1	4	—	—	—
M.Mil.	female	21 yr.	4	—	—	4	—	—	—
D.Mil.	female	4 yr.	—	—	+	+	+	+	+
B.W.	female	21 yr.	5	—	—	1	—	—	1
M.W.	female	4 yr.	6	—	1	—	—	—	—
R.W.	male	5 yr.	7	—	—	—	1	—	—
K.P.	female	9 yr.	8	—	—	1	—	—	—
E.K.	female	22 yr.	9	—	—	—	1	—	—
J.H.	male	9 yr.	10	1	—	—	—	—	—
T.V.	male	11 yr.	11	—	—	—	1	—	—
GGW.	female	14 yr.	12	—	1	—	—	—	—
C.G.	female	12 yr.	13a	—	—	1	—	1	—
S.G.	female	31 yr.	—	—	—	1	—	—	—
G.G.	female	9 yr.	—	—	—	—	1	—	—
M.M.	female	2 yr.	13b	—	—	—	—	1	—
I.B.	male	8/12	15	—	1	—	—	—	—
G.S.	female	13 yr.	16	—	1	2	—	—	—

by the patient M.M. there were 12, while there were only 2 in that presented by the patient I.B. In both cases, they were situated at or near the centre of the lesion or well within the margin. Microscopical examination of scrapings from the various lesions in 10 % caustic potash solution in most cases did not reveal any features of the mycelium which were characteristic, but in the case of the patient I.B. lanugo hair follicle sheaths were observed to be filled with chain mycelium and some large spores, and besides these, there were some dense accummulations of mycelium which suggested the possibility of scutulum formation, and which in fact, led to the discovery of the scutula with the aid of the magnifying glass. It seems possible, in view of this experience, that, in some of the other cases incipient development of scutula may have been overlooked owing to insufficiently detailed clinical examination; indeed, in most cases the correct diagnosis was not arrived at or even suspected until the etiologic agent had been grown in culture.

Solitary scalp lesions were observed in only 3 of the patients; scaling only was present in one of them; in another, crusts were also present; and in the third, the lesion presented was of kerion type. The hair included in the lesions did not exhibit fluorescence when examined in Wood's light. While mycelium, and in one case, patches of arthrospores, were observed on the hair, it was not clear whether or not penetration of the hair by the mycelium had taken place.

Comment.

Although scutulum formation in lesions of the glabrous skin is rarely reported nowadays, (recent reports are those of SCHNEIDER (26) and BLANK (l.c.)), it has often been recorded in past literature. Commencing with QUINCKE (l.c.) the following authors, amongst others, have recorded them: — BODIN (12), WANDEL (27), MEWBORN (l.c.), ADAMSON (28), CHAJES (29), BLOCH (16), PLAUT, cited by PLAUT & GRÜTZ (30) and CONNOR (31). Other names besides these are mentioned by KUMER in JADASSOHN (32). There are only a few records of scalp infection by *T. quinckeanum*, amongst which are those of PLAUT (l.c.), CONNOR (l.c.), SCHMIDT (33), BEINTEMA (34), and recently, BLANK (l.c.). Kerion lesions were described both by SCHMIDT (l.c.) and BEINTEMA (l.c.). Scalp hair invasion was described only by PLAUT (l.c.). According to him, hair thus invaded was indistinguishable from that attacked by *T. schönleini*. BLOCH (16) as already mentioned, described the invasion of a lanugo hair by this dermatophyte, and WANDEL (l.c.) who looked for it in lanugo hair, found mycelium only on the outside of these structures.

Spontaneous infection in kittens by *T. quinckeanum.*

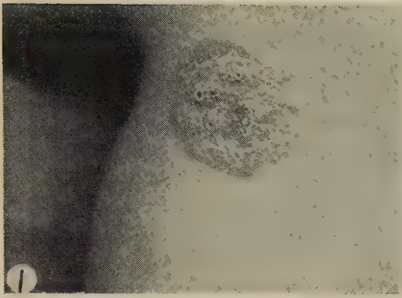
Clinical features of the kitten (M.M.) infected by *T. quinckeanum.* [1])

On admission, this kitten presented with a tumour-like lesion which involved the right eyebrow and right upper eyelid. At a short distance behind it was a large, compound, somewhat convoluted yellow crust. Other lesions observed at the time, were a small depilated area on the bridge of the nose, a lesion with a small yellow crust on the left cheek and another on the inner border of the right ear. These crusts when examined microscopically were found to consist entirely of compacted mycelium and spores. Three days later, the tumour-like lesion above the right eye revealed itself as a large yellow crust 1.0 cm. in diam. Three weeks later, small favic crusts were present along the outer border of the right ear and at the base of the 1st. toe of the right front paw. At 25 days after admission, the number and distribution of favic crusts were as follows: right eyebrow and eyelid (1) Plate II, fig. 1; right ear (3); outer aspect of right elbow (1); inner aspect of right front leg near axilla (2); right hip (1) Plate

---

[1]) Associated with the patient M.M. from whom strain 13b was isolated, was a kitten aged about 4 months. This kitten was traced in a somewhat roundabout manner through information supplied by patients of another infected family (C.G., S.G. and G.G.) from whom strain 13a was isolated. Their lesions were of the circinate type, showing merely a uniform erythema, except for one in which there was some desquamation. They had apparently recently acquired a kitten which in due course had presented with a ,,skin disease". This kitten had been destroyed a few days before these patients' attendance at the skin clinic and was therefore not available for inspection. However, its litter-mate had been given to the family to which the child M.M. belonged and was procured immediately for investigation.

PLATE I



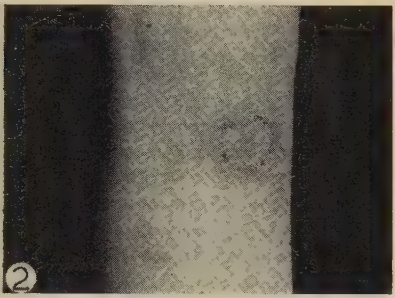
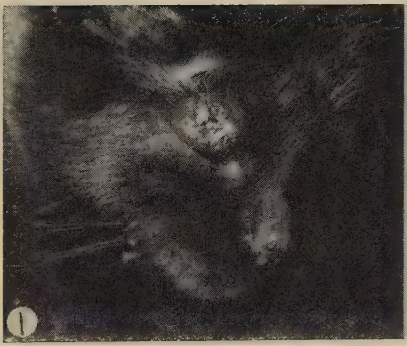


Fig. 1. Lesion with scutula on leg of patient M.M. approx. $^1/_2$ nat. size.
Fig. 2. Lesion without scutula on forearm of patient R.W. approx. $^1/_2$ nat. size.

PLATE II



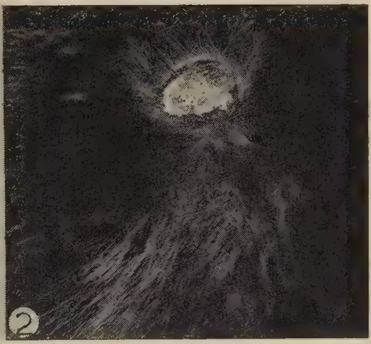


Fig. 1. Head of kitten (M.M.) showing favic crusts on right eyelid and right ear as well as pigmented scar between r. eye and r. ear. approx. $^1/_2$ nat. size
Fig. 2. Favic crust on right hip of kitten (M.M.). approx. $^1/_2$ nat. size.

II, fig. 2; base of 1st. toe right front paw (2); base of 2nd. toe left front paw (2); base of 1st. toe left hind paw (1) and ventral aspect of tail (2). Cursory examination by Wood's light did not reveal any fluorescence in the hair included in the lesions. Many of the crusts incorporated a centrally situated tuft of hair. They were yellowish, circumscribed, circular or elliptical, and flat or slightly concave on their upper surface in most cases, and this was raised above the level of the surrounding skin. They were compact, but crumbled easily on pressure, and were damaged by friction. They measured from 0.4 cm. in diam. on the 1st. toe of the left hind paw to 1.3 cm. by 1.0 cm. on the right eyebrow and eyelid. A feature common to all the lesions was the apparent drawing up around the sides of the favic crust of the skin immediately surrounding it. This skin showed a varying degree of erythema and desquamation but neither was very pronounced. A favic crust, on being detached from the base of the 1st. toe, right front paw, left a raw smooth surface. Spontaneous removal of one of the crusts (that observed on the day of admission, between the right eye and the right ear), left a smooth black scar. (Plate II fig. 1.) Strain 13 c was isolated from the crust situated on the right eyebrow and eyelid.

A second kitten, which belonged to the patient G.S. revealed, on examination, only a few much worn favic crusts on some of the toes of the front and hind paws as well as a few scars on some of the toes. In Wood's light, some of the hairs in the affected areas were yellowish in colour but a green fluorescence was not observed, even on careful examination of the hairs included in the crusts. Microscopical examination of the favic material and hairs revealed much compacted mycelium and spores (presumably oidia developed on the scutulum mycelium) which were closely associated with the hairs, but penetration of the hair was not detected either in preparations mounted in 10 % caustic potash solution or in stained *ex tempore* preparations.

Summary and conclusions.

In the review which serves as introduction to this paper the main results and conclusions of various investigators of "mouse favus" in mice and Man and its etiologic agent *T. quinckeanum* have been set out, and it has been shown that there is much divergence of opinion in their writings. In an endeavour to clarify the various points at issue, investigations have been carried out and have been recorded. In this first part the clinical features of the disease in human patients and in two cats have been described. The majority of the lesions in the human patients occurred on the glabrous skin, scalp lesions being observed in only three patients. The presence of the dermatophyte was noted in hair follicles in the scalp lesions of two of these patients and in a lanugo hair follicle of another patient. Scutula were observed in the solitary lesions of only two patients.

All the lesions examined exhibited varying degrees of inflammatory response. In the two kittens multiple lesions with scutula were present and in one of them were widespread with well-formed favic crusts of large dimensions.

PART II

INOCULATION EXPERIMENTS WITH T. QUINCKEANUM IN ANIMALS.

Experimentally induced infection in kittens with T. quinckeanum.

In order to test the infectivity of the strain 13c which was isolated from the spontaneously infected kitten (M.M.) and in order to be able to follow the clinical course of the disease as it occurs in cats, two female kittens aged between 3 and 4 months were inoculated: one with scutulum material from the spontaneously infected kitten; the other with culture material from a 17-day old Sabouraud glucose slope culture derived originally from the same scutulum material. The anterior aspect of the right and left shoulder in each kitten was inoculated. Prior to this, each kitten was prepared as follows: the hair on the selected inoculation site was clipped close to the skin; the left shoulder was rubbed lightly with sand-paper while the right shoulder was not so treated; each inoculation site was then wetted with sterile water and the inoculum rubbed in with a loop. In the case of the culture inoculum, a little of the culture medium was included.

Results and conclusions.

The main results of this experiment are set out in Table II. Some of them are noteworthy. Thus, it seems that it is unnecessary to abrade the skin of the inoculation site in order to ensure successful infection in young kittens although by so doing the incubation period is shortened. Also, scutula (or favic crusts) which occur in experimentally induced lesions are quite as well developed and quite as large as those which result from spontaneous infection. Lastly, spread of infection from the site of inoculation does not seem to occur, whereas this appeared to be the case in the spontaneously infected kittens (M.M. and G.S.).

Re-inoculation of kittens with T. quinckeanum.

Two weeks after all traces of infection seemed to have disappeared from kitten No. 2, both kittens were re-inoculated with a 14-day old Sabouraud glucose slope culture of strain 13c in order to ascertain if any degree of immunity had been acquired as a result of

TABLE II.

Experimentlly induced infection in kittens

Kitten No. 1

Site of inoculation	Reaction			
	4 days	7 days	12 days	16 days
L. shoulder	scaling and crusting	Favic crust 1.0 × 0.6 cm	Favic crust 1.4 × 1.4 cm	Favic crust 1.4 × 1.4 cm
R. shoulder	N. A. D.	N. A. D.	Rough spots	Broken up crust 1.5 cm
	21 days	26 days	30 days	33 days
L. shoulder	Favic crust 1.4 × 1.2 cm satellite 0.6 cm	serous crusts only	serous crust	clear
R. shoulder	4 favic crusts with tufts of hair	2 small contiguous favic crusts	Favic crust	Favic crust

Kitten No. 2

Site of inoculation	Reaction			
	4 days	7 days	12 days	16 days
L. shoulder	slight scaliness	marked scaliness	Favic crust 1.0 × 1.8 cm	Favic crust 1.5 × 1.5 cm plus satellites
R. shoulder	N. A. D.	Rough spots	Roughened surface	cluster of 5 favic crusts 0.4 × 0.6 cm
	21 days	26 days	30 days	33 days
L. shoulder	4 worn favic crusts	3 favic crusts	peripheral row of small favic crusts	2 favic crusts 1.0 × 0.5 cm 0.5 × 0.5 cm
R. shoulder	Raw patch	Raw patch	clear	clear

the previous infection. Concurrently, a third kitten was inoculated with the same culture in order to check its pathogenicity. The sites selected for inoculation were the right shoulder and the right hip in each kitten, the left shoulder and the left hip being reserved as controls. Again the hair of the site selected for inoculation was clipped closely to the skin, and the sites wetted with sterile water. The control sites were prepared likewise. On this occasion, abrading of the skin with sand-paper was omitted as it was considered to be unnecessary in the light of the results of the previous experiment.

Results and Conclusions.

At 5 days, erythema and slight desquamation were apparent in the inoculation sites, but a favic crust had begun to develop only in the inoculation site on the shoulder of No. 3 kitten (inoculated for the first time). It was trough-like and centred around a small accidental scissors nick made during clipping. At a later date, in this same kitten, after the favic crust had been spontaneously rubbed off, 3 other small crusts developed on the same inoculation site. At about 8 weeks after it had been inoculated this kitten exhibited a 0.4 cm. diam. favic crust on the control site also, this spread of infection being doubtless due to handling during examinations. This crust eventually reached a diameter of 1.0 cm. The inoculation sites on the right hip of all three kittens exhibited only some matted scales. The right shoulder lesions of the reinoculated kittens did not show any increased reaction or further features of note after the initial reaction observed at 5 days. It thus seems that one infection by this dermatophyte confers at least some degree of immunological response in kittens, but of course increasing age may also be a determining factor in resisting the infective process.

Inoculation of kittens with T. quinckeanum in order to ascertain if infected hair fluoresced in Wood's light.

Unfortunately, the possibility that infected cat's hair might fluoresce when exposed to Wood's light had not been seriously considered until after VANBREUSEGHEM'S paper (2) had come to the present author's notice. The opportunity to observe it during the experiments described above was consequently missed. When however, due note had been taken of VANBREUSEGHEM'S observation that, on exposure to Wood's light a green fluorescence was elicited from guinea-pig hair infected by *T. quinckeanum*, this was sought for and abundantly confirmed by the present author as recorded later in this article. Thereupon it was resolved to seek it in cat hair infected by *T. quinckeanum*. For this purpose, 4 kittens from the same litter, aged between 4 and 5 weeks, were prepared and inoculated as follows: the hair on the right and left axillae was clipped closely to the skin and wetted with sterile water; (in one kitten, No. 1, to make doubly sure of infection taking place, the skin of the inoculation site was slightly abraded with sand-paper.) A 3-week old subculture served as the inoculum. Only the right axilla was inoculated, the opposite one being reserved as the control. Kittens No. 1 and 2 were inoculated with strain 13c and kittens No. 3 and 4 with strains 7 and 8 respectively.

Results and conclusions.

At 3 days, the inoculation site in kittens No. 1 and 2 exhibited some erythema and desquamation; in No. 4, some desquamation and in No. 3 no reaction. At 8 days, the hair showing above the skin surface was still negative in Wood's light in all cases. Unfortunately, all the kittens had by this time become intercurrently infected by what was thought to be a pneumonia-type virus and died shortly after this within a few days of each other. At 8 days, No. 4 kitten was dead, and as No. 2 was found to be *in extremis* its life was terminated. In No. 2 kitten (inoculated with strain 13c) mycelium was abundant in the scales of the inoculation site, but was not observed to have penetrated the hair, whereas in No. 4 it was present not only in the scales but also to some extent apparently in the hair. In both these kittens, the hair in the inoculation site was negative in Wood's light. At 10 days, No. 1 kitten died. The hair in the inoculation site of this kitten exhibited a bright green fluorescence in Wood's light and when some fluorescent hairs were examined microscopically they were found to be invaded by mycelium and surrounded by a sheath of arthrospores measuring when mounted in water, 3.8 μ to 5.7 μ in diam. At 11 days, the last of the kittens, No. 3, died, but although the scales in the inoculation site were found to be heavily invaded by mycelium, invasion of the hair was not observed and no fluorescence detected in it by Wood's light. It is felt nevertheless, that, had all these kittens survived for a longer period, all would have sustained invasion of the hair in the inoculation site, and accordingly, this hair, as in the case of kitten No. 1, would have been, in due course, positive in Wood's light. It thus seems that at least in experimentally infected kittens, the hair fluoresces a green colour in Wood's light as a result of invasion by the mycelium of *T. quinckeanum.*

Artificially induced infection by T. quinckeanum in mice.

As no spontaneously infected mice were available for investigation, it was necessary to induce infection in laboratory mice in order to test the ability of some of the strains of *T. quinckeanum* isolated from patients, to infect mice and produce scutula on them. 1-month old mice were accordingly prepared and inoculated as follows: after clipping and slightly abrading with sand-paper a site on the skin of the back at a little distance from the root of the tail, a small amount of a 25-day old Sabouraud glucose agar slope culture was rubbed in with a little sterile water. 3 mice were used to test each strain. The strains which were at first tested were 6, 9 11 and 12, all of which had been isolated from human lesions of the tinea circinata type *sine scutulis.*

Results.

At 4 days, in the case of mice inoculated with strains 9, 11 and 12, the inoculation site was rough and crusty, and in strain-9 mouse a small scutulum was already present. At 6 days, an elongate, trough-like yellow favic crust was observed in two of the strain -11 mice and an irregularly-shaped one in the third; in two of the strain-12 mice a large favic crust was also present, and a crater-like crust had developed in two of the strain-6 mice. At 6 days, scutula in the strain-11 mice measured from 0.5 — 1.2 cm. long by 0.25 cm. wide; in the two strain-12 mice which were affected they measured from 0.75 cm. — 1.0 cm. long by 0.5 — 0.75 cm. wide. In the strain-6 mice they were much smaller. The scutulum observed at 4 days in one of the strain-9 mice had been rubbed off or nibbled off. In all cases the upper surface of the favic crust was raised above the level of the surrounding healthy skin to a height of about 1—2 mm. but the skin in the immediate vicinity was drawn up against the sides of the crust as was observed in the case of the spontaneously infected kittens.

At a later date, strain 13 c which was isolated from scutulum material on the kitten (M.M.) was tested on 4 white mice which were prepared as previously described; two of them were inoculated with scutulum material obtained from the kitten (M.M.) and two with a 17-day old Sabouraud glucose slope culture.

Results.

At 4 days, some scaling and crusting were observed in the mice which were inoculated with culture material whereas those inoculated with scutulum material were not affected. At 6 days, one of the mice inoculated with culture material was dead; its scutulum measured 0.4. cm by 0.15 cm. and was trough-like in shape. A small scutulum was present on the other mouse and measured 0.15 cm. in diam.; this was absent on the following day, having doubtless been rubbed off. No reaction was observed at this stage in the inoculation sites of the mice inoculated with scutulum material.

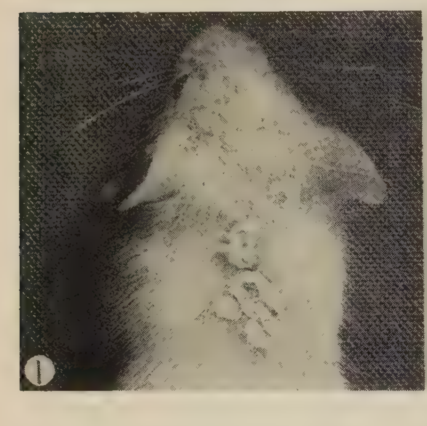
Fluorescence of infected mouse hair in Wood's light.

In order to ascertain if mouse hair infected by *T. quinckeanum* fluoresced a green colour in Wood's light, six 1-month old white mice were obtained and prepared as before by clipping the hair of the site selected for inoculation and abrading the skin slightly with sand-paper. On this occasion, the site chosen for inoculation was the skin at the back of the neck as it was thought that a lesion developing there would not be interfered with. 1-week old honey-peptone agar slope cultures were used as the inoculum and the strains of *T. quinckeanum* tested were: — numbers, 7, 8, 11, 12 and 13 b, (that isolated from the patient M.M.); the sixth mouse served as control. The inoculum was prepared by pipetting some sterile water into the culture tube and rubbing into it some of the surface growth on the slope with a sterile spatula. A drop of the resulting suspension was then smeared over and rubbed into the inoculation site

Results.

At 2 days, all the inoculated mice exhibited some slight reaction at the inoculation site; and at 4 days, this was marked, but varied from a white scurfiness to crusting which was golden yellow in Wood's light; at 6 days, favic crusts (Plate III fig. 1) had developed in the inoculation sites of all the mice, and were a pale golden yellow by Wood's light; at 8 days, Wood-positive (green) hairs were observed in the mice inoculated with strains 11, 13b and 14 respectively, and at 12 days, they were observed in the inoculation sites of all the mice. Penetration of the fluorescent hair by the mycelium was confirmed in all cases microscopically. The mice were then killed and their inoculation sites preserved in formol-saline.

PLATE III



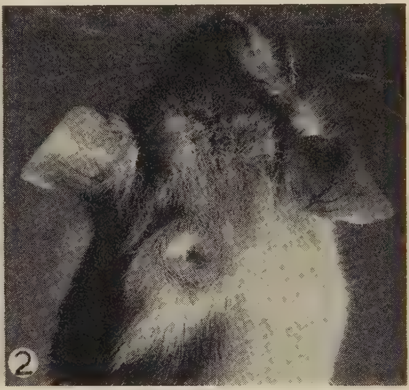


Fig. 1. Mouse with favic crusts of strain 7 at twelve days after inoculation. Approx. $^3/_4$ times nat. size.

Fig. 2. Mouse with scutulum of strain 16 at fifteen days after inoculation. Approx. $^3/_4$ times nat. size.

At a later date, the more recently isolated strains of *T. quinckeanum* (strains 15 and 16) were tested in the same way. In strain 15 the results obtained were similar to those previously obtained, and in the case of strain 16 a scutulum was developed which was so perfectly formed that when at 15 days it had reached a diameter of 0.5 cm. the lesion bearing it was excised *post mortem* and preserved in formol saline without a check having been made for invasion of the hair by mycelium or fluorescence in the hair by Wood's light. (Plate III, fig. 2).

Conclusions re. experimentally induced infection in mice.

The formation of favic crusts or scutula occurs in young mice experimentally infected with *T. quinckeanum* strains isolated from circinate lesions *sine scutulis* of the human skin, and also with a strain isolated from the scutulum of a spontaneously infected cat. Penetration of the hair by mycelium takes place, and such hair fluoresces a green colour in Wood's light.

Experimentally induced infection with T. quinckeanum in guinea-pigs.

In order to confirm the observation made by VANBREUSEGHEM (l.c.) that guinea-pig hair when infected by *T. quinckeanum* fluoresced a green colour in Wood's light, a 4-week old guinea-pig was inoculated with a 1-week old Sabouraud glucose slope culture of strain 13b after sites on the right and left hip respectively had been prepared for the purpose. The site on the right hip was slightly abraded with sand-paper while that on the left hip was not so treated. A control guinea-pig was similarly prepared.

Results.

At 5 days, a ridge measuring about 2 cm. long by 0.25 cm. wide was observed on the lower periphery of the lesion which had developed on the right hip. The hairs were Wood's negative, and when examined microscopically were intact although surrounded by mycelium; at 9 days, hairs on both right and left hips exhibited a green fluorescence within the inoculation sites; at 11 days, the crust which had formed in the inoculation site on the right hip had been rubbed off or nibbled off, and could not be recovered. The lesion on the left hip was excised *post mortem*, and preserved in formol-saline.

At a later date, strains 6, 8, 11, 12, and 14 (isolated from a kitten in Nottingham and kindly provided by Mr. P. AUSTWICK) were tested for infectivity and production of scutula in guinea-pigs as well as for their ability to penetrate guinea-pig hair and cause it to fluoresce in Wood's light. On this occasion, the site selected for inoculation was the skin on the right hip. This was prepared as before by clipping the hair and slightly abrading the skin. The inoculum used was a suspension of spores and mycelium from a 9-day old Sabouraud glucose slope culture prepared as described previously in connection with the test for fluorescence in mouse hair infected by this dermatophyte. Two similarly prepared, but not inoculated, guinea-pigs served as controls.

Results.

At 5 days, all the inoculated guinea-pigs showed slight hair disturbance at the inoculation site; at 7 days, a reaction was observable in all of them; this varied from roughness or slight scaliness to matted scales and crusting. (At this stage, collars made of tire-tubing as used by Kaplan & Georg (36) were fitted around the necks of these guinea-pigs to prevent them from interfering with the lesions which had developed). At 11 days, thick yellow crusts had formed a more or less continuous layer on the surface of the lesion in all the guinea pigs except that inoculated with strain 8 in which only a white crust had formed. These crusts were not similar in appearance to those previously described as occurring in the infected mice and kittens, being a mixture of epithelial débris, inflammatory exudate and mycelium. At 9 days, green fluorescence in Wood's light was observed in the hair of the inoculation site in guinea-pigs with strains 8, 11, 12 and 14 respectively, and at 11 days in the hair of the inoculation site of the guinea-pig inoculated with strain 7. Penetration of the fluorescent hair by mycelium was confirmed in all cases in stained preparations.

Conclusions re. experimentally induced infection in guinea-pigs,

The hair of guinea-pigs infected experimentallyby *T. quinckeanum* is penetrated by the mycelium of this dermatophyte and exhibits a green fluorescence in Wood's light as a result of this. The local reaction resulting from infection is severe and tends to mask scutulum formation.

Retroculture in the foregoing inoculation experiments.

Retroculture was carried out in all cases where positive results were obtained, the organism introduced by inoculation being thereby recovered in each case.

Summary and conclusions.

Inoculations with native material and cultures of *T. quinckeanum* have been carried out on kittens and mice, and with cultures only on guinea-pigs.

The results show, on the whole, that all strains of this dermatophyte which were used were capable of infecting these animals and that all types of animal used responded to primary infection by producing lesions with scutula, but that in the case of the guinea-pigs the scutula, which were in an incipient stage of development, were masked by excessive inflammatory reaction. They also show that penetration of hair by this dermatophyte takes place during its growth as a parasite and that as a result of it such hair fluoresces a green colour in Wood's light. Finally, reinoculation of previously infected kittens results in abortive lesions in which scutula are not formed and the inflammatory response is of short duration.

Acknowledgement.

The author gratefully acknowledges the cooperation of his clinical colleagues in referring to him patients for investigation; in particular that of Dr. J. T. INGRAM outgoing Head of this Department and now Professor of Dermatology at the University of Durham, to whom he is also indebted for helpful suggestions and criticism; and that of Drs. F. F. HELLIER and S. T. ANNING, consultants. He thanks Dr. K. ZINNEMAN of the Department of Bacteriology for his kind help with German texts. In addition, his thanks are due to the staff of the Disinfestation Department of the Public Health Service for obtaining animals for examination; to clinical photographers Mr. R. PEGG and Miss B. WALSH; and lastly, but especially, to Mr. R. FORSTER (technician) for much help in connection with laboratory work and the care of the aminals investigated and also the cooperation of Mr. G. R. OURY, Superintendent of the Experimental Animal department.

References

1. BENNET, J.: 1850. On the vegetable nature of tinea favosa. J. med. Sc. London & Edinburgh. **11**: 40.
2. VANBREUSEGHEM, R. 1950. Position systématique et nomenclature de l' *Achorion Quinckeanum.* Ann. Parasitol. **25**: 188—199.
3. GLUGE & D'UDEKEM, J.: 1857. De quelques parasites végétaux développés sur des animaux vivants. Bull. Acad. Roy. Sci., Lettres Beaux-Arts Belgique. 26e ann. 2e. ser. **3**.
4. BAZIN: Leçons sur les affections cutanées parasitaires de Bazin. 2nd. ed. Paris. 1862.
5. SAINT-CYR, F.: 1869. Etude sur la teigne faveuse chez les animaux domestiques. Ann. Dermatol. Syphil. **1**: 260—286.
6. FRIEDREICH, N.: 1858. Favus bei der Maus. Arch. Pathol. Anat. Physiol. klin. Med. **3**: 287.
7. QUINCKE, H.: 1887. Ueber Favuspilze. Arch. Pathol. Pharmakol. **1** & **2**.: 62—76 (delivered in 1886).
8. SABOURAUD, R.: Les Teignes. Masson & Cie. Paris.: 543, 1910.
9. BODIN, E.: 1907. Sur un nouveau champignon du favus: *Achorion gypseum.* Ann. Dermatol. Syphil. **8**: 585—602.
10. BLOCH, B.: 1911. Das *Achorion violaceum,* ein bisher unbekannter Favuspilz. Dermatol. Z. **18**: 815.
11. SABOURAUD, R., SUIS, & SUFFRAN,:1909. La „crête blanche" et son parasite: *Achorion gallinae.* Rev. Vet. Toulouse. Oct/Nov.
12. BODIN, E.: 1902. Sur le champignon du favus de la souris. (*Achorion quinckeanum*). Arch. Parasitol. **5**: 5—30.
13. SABOURAUD, R.: 1928. Généralités concernant les dermatophytes. Ann. Dermatol. Syphil. ser. 7. **9**: 656—669.
14. SABOURAUD, R.: 1931. „Eugène Bodin" 1886—1931. Ann. Dermatol. Syphil. ser. 7. **2**: 829—832.
15. TOMASCEWSK , E.: 1911. Kulturelle und experimentelle Untersuchungen über *Achorion Schönleini* und *Achorion quinckeanum.* Dermatol. Z. **18**: 887-903.
16. BLOCH, B.: 1911. Über das Vorkommen des Mäusefavus beim Menschen und seine Stellung im System der Dermatomykosen. Dermatol. Z. **18**: 451—461.
17. ZOPF, W.: Die Pilze in morphologischer, physiologischer, biologischer und systematischer Beziehung: 481—482, 1890. Eduard von Trewendt. Breslau.
18. BLANCHARD, R.: 1896. Parasites végétaux. In Bouchard, C. Traité de Pathologie générale. **2**: 894—896.

19. OTA, M & LANGERON, M.: 1923. Nouvelle classification des dermatophytes. Ann. Parasitol. **1**: 305—336.
20. LANGERON, M. & MILOŠEVIĆ, S.: 1930. Morphologie des dermatophytes sur milieux naturels et milieux à base de polysaccharides. Essai de classification. Ann. Parasitol. **8**: 465—508.
21. GUIART, T. & GRIGORAKI, L.: 1928. La classification botanique des champignons des teignes. Lyon Med. **141**: 369—378.
22. CONANT, N. F., SMITH, D. T., BAKER, R. D., CALLAWAY, J. L. & MARTIN, D. S. Manual of clinical mycology. 2nd. ed.: 336, 1954. W. B. Saunders Co. Philadelphia.
23. Medical Research Council of Gt. Britain, Medical Mycology Committee, Memorandum No. 23. Revised ed.: Nomenclature of Fungi pathogenic to Man and animals: 9, 1958.
24. MACLEOD, J. & MUENDE, I.: Practical handbook of the pathology of the skin. 2nd. ed.: 383, 1940. H.K. Lewis & Co. Ltd.
25. SKINNER, C. E., EMMONS, C. W., & TSUCHYA, H. M.: Henrici's Molds, Yeasts and Actinomycetes.: 161, 1948. John Wiley and Sons, Inc., New York.
26. SCHNEIDER, W.: 1954. Favusepidemie durch Feldmäuse. Der Hautarzt. 8: 348—341.
27. WANDEL, O.: 1903. Zur Frage des Thier- und Menschen-favus. Dtsch. Arch. klin. Med. **76**: 521—543.
28. ADAMSON, H.: 1909. Favus of the glabrous skin, with cultures of the type known as *Achorion Quinckeanum* of Bodin. Proc. R. Soc. Med. (Dermat. Sect.) **2**: 1– 3.
29. CHAJES, B. VON: 1910. Ueber das Vorkommen von Mäusefavus beim Menschen. Med. Klin. **1**: 142—143.
30. PLAUT, H. & GRÜTZ, O.; in KOLLE, W. & WASSERMAN, A. VON: Handbuch der Pathogenen Mikroorganismen. (Die Hyphenpilze oder Eumyceten) **5**: 225, 1928. Gustav Fischer und Urban & Schwarzenber/Berlin and Vienna.
31. CONNOR, J.: 1932. Favus in mice and men. Med. J. Austr. No. 26 **2**: 765—767.
32. KUMER, L.: Dermatomykosen; in Jadassohn, J.: Handbuch der Haut- und Geschlechtskrankheiten. **2**: 666, 1928. Julius Springer, Berlin.
33. SCHMIDT. P.: 1932. Über die Pilzflora Westfalens insbesondere des Münsterlandes. Arch. Dermatol. **167**: 418—447.
34. BEINTEMA, K.: 1933. Klinische und kulturelle Beobachtungen bei *Achorion Quinckeanum*. Dermatol. Z. **68**: 21—27.
35. QUINCKE, H.: 1889. Doppelinfection mit Favus vulgaris und Favus herpeticus. Monatsh. prakt. Dermatol. **8**; No. 2: 49—51.
36. KAPLAN, W. & GEORG, L. K.: 1957. A device to aid in the development of mycotic and other skin infections in laboratory animals. Mycologia. **49**: 604—605.

MOUSE FAVUS DUE TO TRICHOPHYTON QUINCKEANUM (ZOPF) MAC LEOD & MUENDE: A REAPPRAISAL IN THE LIGHT OF RECENT INVESTIGATIONS. III.

Charles J. La Touche, L. A. H. (Dubl.), M.Sc., M.I. Biol.

(*From the Department of Dermatology, the General Infirmary at Leeds, Yorks, England and the Department of Bacteriology, University of Leeds.*.

(11.VII.1959)

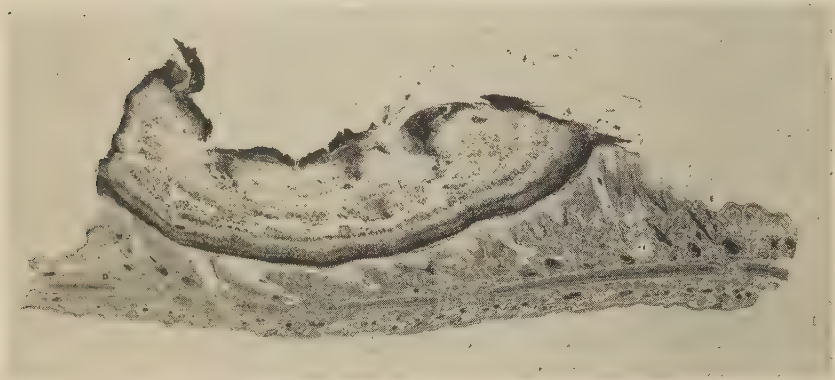
Histopathology of scutulum-bearing lesions in animals.

Necropsy sections from spontaneously infected kitten (M.M.)

Necropsy sections from material fixed in formol-saline were stained by P.A.S. (Hotchkiss-McManus modification as used by Kligman & Mescon (1950) and counterstained by Auber's (1950) "Sacpic" and haematoxylin, as used by Sellers, Sinclair & La Touche (1956), and described by Forster (in Press).

The contour of the necropsy sections, on the side which included the lesion, showed the scutulum lying in a concavity of the epidermis between two peaks which reached a height well above the surface level of the surrounding healthy tissue, the thickness of the section being greatest beneath these peaks. (Plate I and Plate II, fig. 1). The sides of the scutulum were flanked to a varying degree by the layers of the epidermis: while beneath the scutulum, owing doubtless to downward pressure by the developing scutulum, some decrease in the width of the connective tissue was observable, particularly in the ear sections. (Plate II). In all cases the scutulum was strongly Schiff positive. It consisted entirely of fungus material except at its periphery where there was some infiltration by leucocytes. The major portion nearest to the underlying skin was composed of zonated, densely interlaced, branched, septate mycelium alternating with less dense mycelium of a similar type. The direction of growth was in the main perpendicular to the surface of the skin. In fully developed scutula, the distal portion was less densely compacted and had formed chains of elliptical spores (oidia) many of which had become free. Young scutula and the younger peripheral parts of older scutula were roofed over by stratum corneum which was infiltrated by inflammatory exudate. (Plate I). The floor or innermost part of the scutulum was in contact with the innermost layers of the stratum corneum or, in some cases, with the outermost layers of the stratum Malpighii, but was also frequently separated from these tissues by a pool of inflammatory exudate which included many polymorphonuclear leucocytes. (Plate III, fig. 1.)

Plate I.



Section through r. ear of kitten (M M.) showing contour of lesion and young scutulum covered by stratum corneum infiltrated by inflammatory exudate. P.A.S., "Sacpic" and H. X 9.

It was mentioned earlier (Part I) that many of the favic crusts observed during clinical examinations of the infected kitten (M.M.) incorporated a tuft of hair. In the necropsy sections, some of these were seen to be extensively invaded by mycelium, as was the surrounding intrafollicular stratum corneum. The hyphae had grown downwards on the outside of the hair-shaft and within it; in some cases parallel growth was a marked feature of these hyphae; in others a network. The hyphae themselves had, in some of the hairs, and on the epithelial lining of the follicle, formed chains of arthrospores in the manner characteristic of other *Trichophyton* species in the hair follicle; moreover, in some of these hairs, the medulla was filled with relatively large spores (2.8 μ—5.7 μ in diam.) while the space between the hair-shaft and the follicular wall was filled with spores reaching equally large diameters and extending upwards into the scutulum. Short chains of spores arising from chain mycelium within the follicular epithelium suggested that the spore-sheath originated from this mycelium and not from mycelium situated on or in the hair-shaft. This mode of origin has already been postulated by Sellers, Sinclair & La Touche (loc. cit.) in calf hair infected by *Trichophyton verrucosum* var. *discoides*. The mycelium situated in and on the hair and that in the follicular sheath was quite distinguishable from that of the scutulum by its mode of growth.

Plate II.

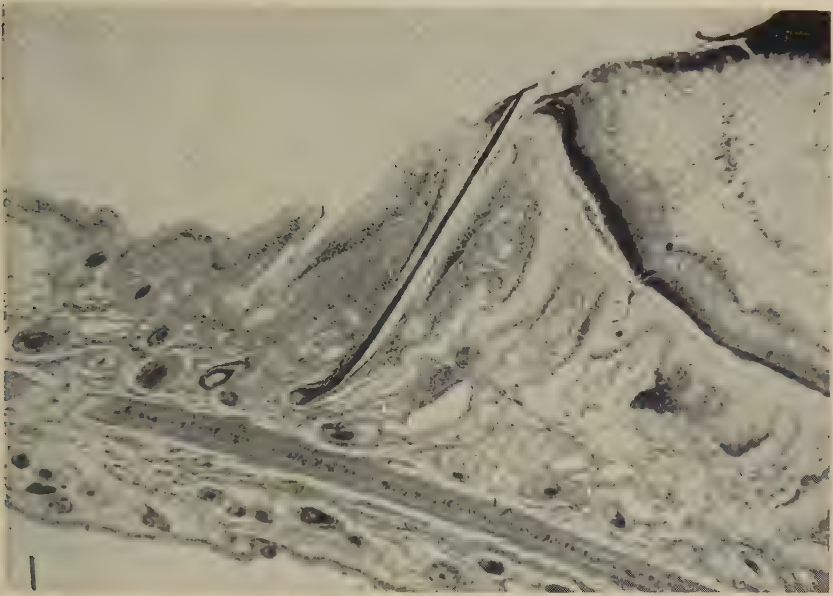


Fig. 1. Section through r. ear of kitten (M.M.) showing tissue reaction in scutulum-bearing lesion as well as zonation of scutulum.

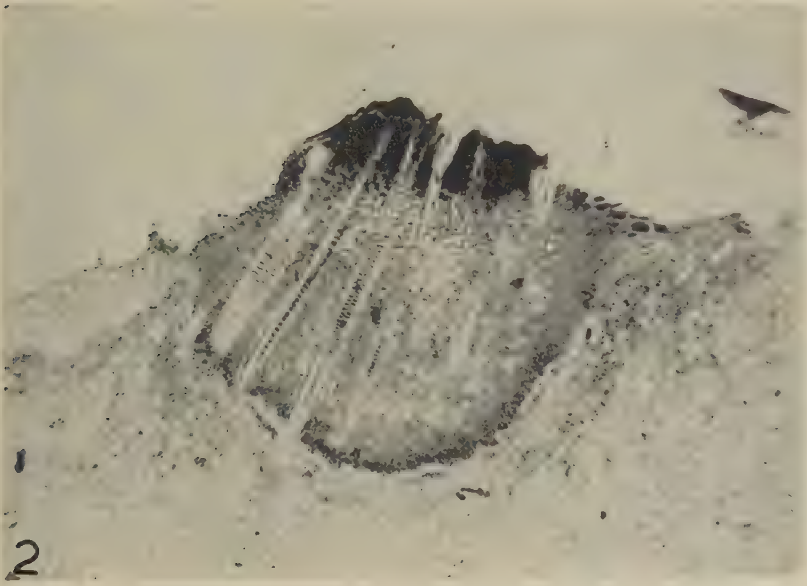


Fig. 2. Section through peripheral part of scutulum of strain 12 at 12 days after inoculation of female mouse skin, showing overlying stratum corneum infiltrated by inflammatory exudate. (Section cut parallel to width of scutulum). P.A.S., "Sacpic" and H. × 120.

Plate III.

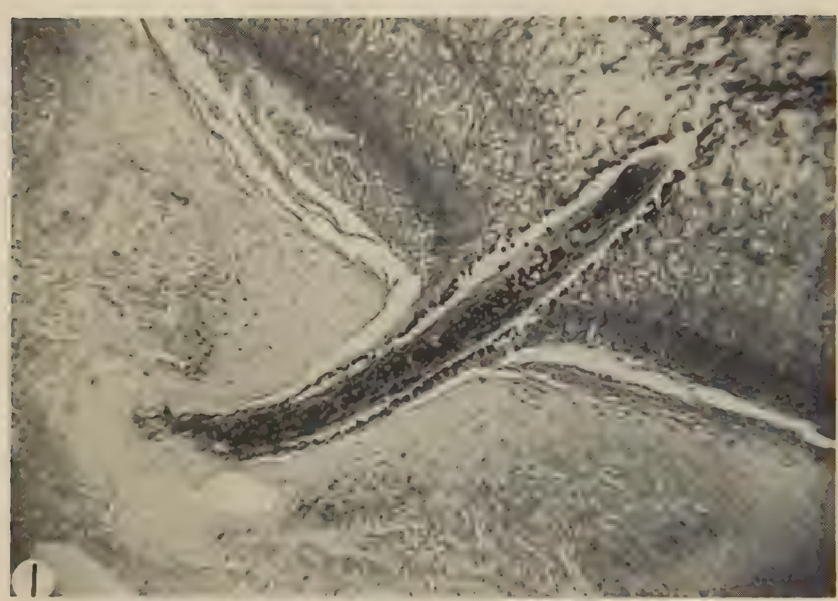


Fig. 1. Section through lesion in r. ear of kitten (M.M.) showing infected hair follicle and hair shaft at base of scutulum. P.A.S., "Sacpic" and H. × 200.

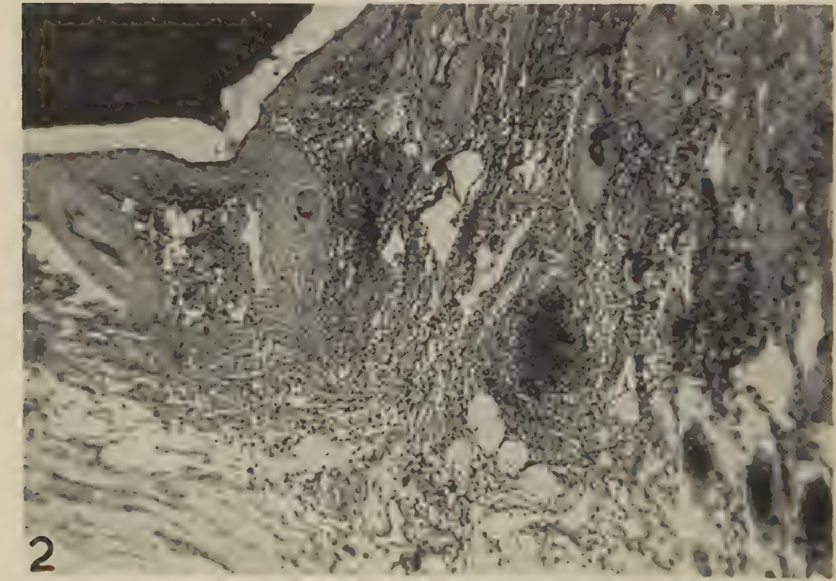


Fig. 2. Section through lesion in male mouse skin at twelve days after inoculation with strain 12 showing general inflammatory reaction which includes several perifollicular micro-abscesses. Part of scutulum shown at top left. P.A.S. "Sacpic," and H. × 200.

The tissue reaction: oedema, engorgement of capillary blood-vessels, infiltration of the dermis and epidermis as well as increased thickness of the stratum Malpighii, was conspicuous in the peripheral zone of the lesion bordering on the scutulum as well as in the intervening zone situated beneath the scutulum. (Plate I and Plate II, fig. 1) Micro-abscesses which could be seen in some of the sections were clearly perifollicular. (Plate IV, fig. 1).

Necropsy sections of experimentally induced lesions in mice.

As no spontaneously infected mice were available for the study of the histopathology of lesions in mice caused by infection due to *T. quinckeanum*, necropsy material obtained from the lesions of the previously mentioned experimentally infected mice was used. This material was fixed and stained by the same procedures as were followed in the case of the kitten necropsies.

Much the same features were observed in sections of this material as were observed in the kitten lesion sections, although obviously on a smaller scale. Moreover, it was abundantly clear, that here also some of the hair-shafts were extensively invaded by this dermatophyte; that many of the hyphae had formed chains (Plate IV, fig. 2.) and that spore sheaths were present in some of the follicles. The structure and development of the scutulum agreed in their essential features with what was observed in the kitten lesion necropsies, and the tissue reaction, though variable, was also, by and large, of the same type. (Plate II, fig. 2. Plate III, fig. 2. and Plate V, fig. 1)

Necropsy sections of experimentally induced lesions in guinea-pigs.

Necropsy sections from material fixed and stained as previously described showed the following main features: Scutulum formation was in an incipient stage and was moreover masked by the hyperkeratosed stratum corneum which was heavily infiltrated by inflammatory exudate and polymorphonuclear leucocytes (Plate V, fig. 2.) Where it occurred, the opening of the hair follicle was much dilated and filled with compacted mycelium which grew downwards in the follicular epithelium, in some cases sparing the hair-shaft, but in others penetrating it. Chain mycelium in the follicular epithelium and in the space between it and the hair-shaft was observable as were also spore sheaths around the hair shaft and spores within the medulla of some of these. The dermis was oedematous in parts and heavily infiltrated with inflammatory exudate and polymorphonuclear leucocytes; engorgement of capillaries and perifollicular abscesses were also a conspicuous feature in these sections.

Plate IV.

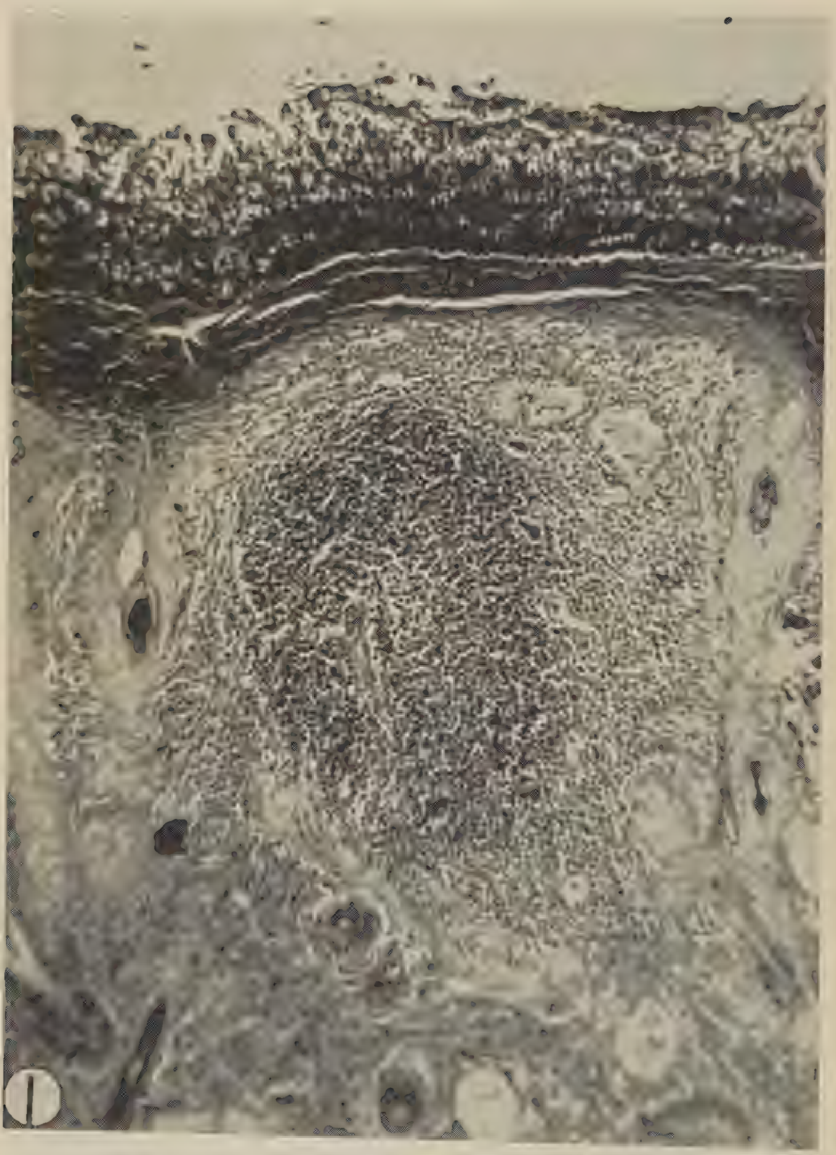


Fig. 1. Section through lesion on r. hip of kitten (M.M.) showing part of mature scutulum and perifollicular micro-abscess in underlying dermis. P.A.S. "Sacpic" and H. × 200.

Plate IV.

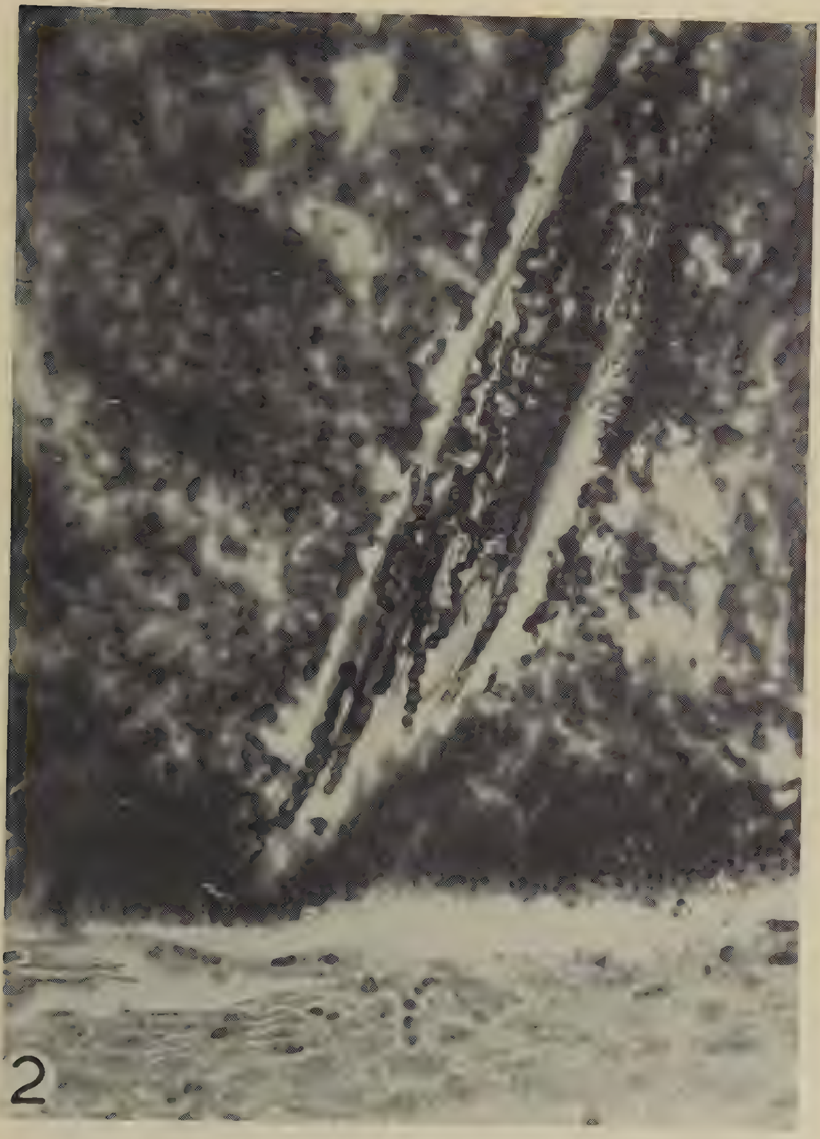


Fig. 2. Section through base of scutulum of strain 12 in female mouse skin lesion showing chain mycelium in and on hair shaft. P.A.S., "Sacpic" and H. × 514.

Plate V.

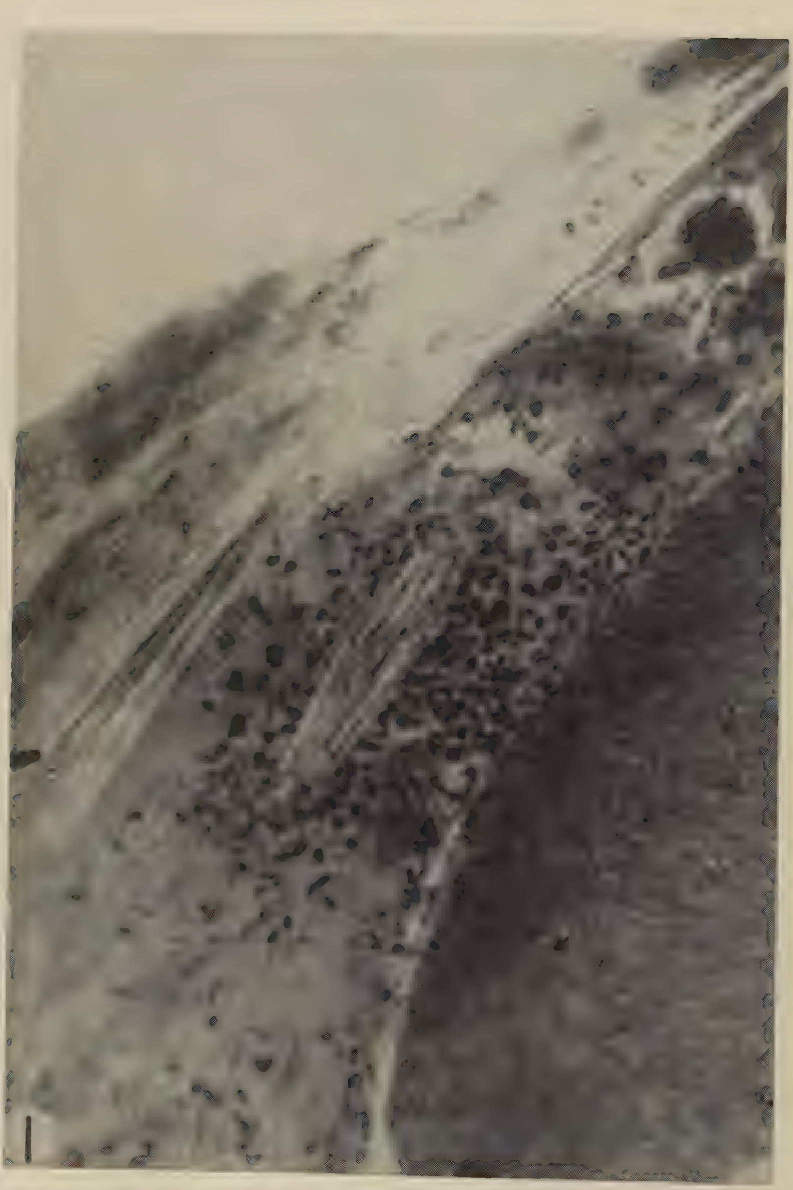


Fig. 1. Section through lesion in female mouse skin at periphery of scutulum of strain 12 showing incipient stage of perifollicular abscess formation. P.A.S., "Stepic" and H. × 514.

Plate V.

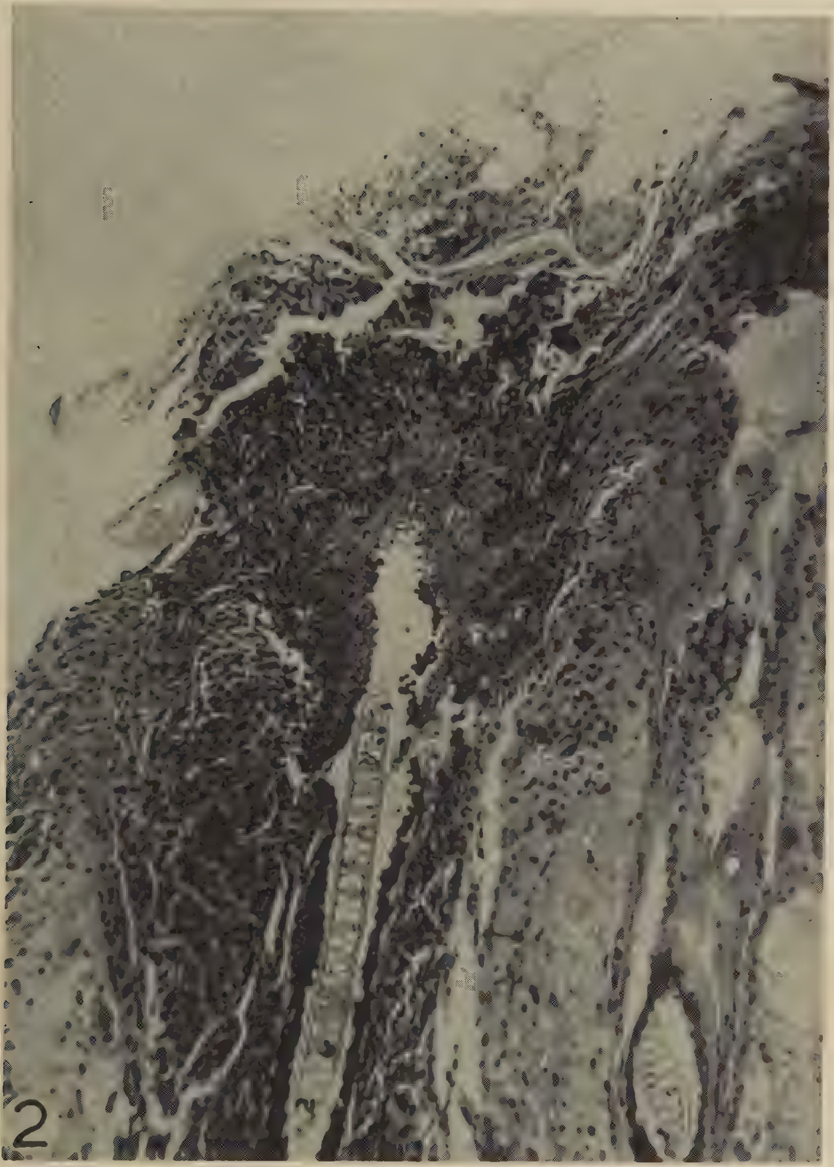


Fig. 2. Section through lesion in female guinea-pig showing initial development of scutulum of strain 13b at 11 days after inoculation. Scutulum heavily infiltrated by inflammatory exudate and polymorphonuclear leucocytes. P.A.S. "Sacpic" and H × 200.

Summary and conclusions.

Sections of necropsy material of scutulum-bearing lesions from a kitten spontaneously infected by *T. quinckeanum* were stained by P.A.S., Sacpic and haematoxylin to reveal various features of the scutulum and the reaction which the parasite provoked in the tissues of the host.

Such sections showed that the scutulum and other fungal structures were strongly Schiff positive.

They also showed that, although the parasite was confined to the outermost layer of the epidermis (stratum corneum) it was also capable of invading the hair shaft and inner follicle sheath and of producing arthrospores both within the hair shaft and outside it (ecto-endothrix).

The main inflammatory response of the host to the invasion of this organism showed itself in the massive infiltration of the dermal and epidermal tissues in the affected areas by serous exudate and polymorphonuclear leucocytes; other features being the undermining of the scutulum by pools of pus, the formation of discrete perifollicular microabscesses in the dermis, the engorgement of capillaries and oedema and lastly the parakeratosis.

Necropsy sections from lesions of mice likewise experimentally infected by this dermatophyte showed much the same features as those found in the necropsy material of the spontaneously infected kitten.

Necropsy sections from lesions of guinea-pigs experimentally infected by this organism showed incipient scutulum formation much infiltrated by inflammatory exudate and polymorphonuclear leucocytes. The dermis was also heavily infiltrated and showed perifollicular-abscesses. These sections also revealed invasion of the hair follicles and hair shaft by the dermatophyte.

Acknowledgement.

The author gratefully acknowledges the painstaking work of his technician, Mr. R. A. Forster in the preparation of the necropsy sections which formed the basis for the descriptions and illustrations contained in this part of the author's account of his investigations. His thanks are once more due to the clinical photographers at the Leeds General Infirmary for processing the photomicrographs with the exception of that reproduced in Plate I which was taken and processed by Mr. L. Jepson, technician at the School of Dentistry, University of Leeds.

References.

Kligman, A. M. & Mescon, H., (1950). The periodic acid-Schiff stain for the demonstration of fungi in animal tissues. J. Bact. **60:** 415—421.

Auber, L. (1950). The anatomy of follicles producing wool-fibres with special reference to keratinization. Trans. Roy. Soc. Edin. **67:** 1, 7 : 191—254.

Sellers, K. C., Sinclair, W. B. & La Touche, C. J. (1956). Preliminary observations on natural and experimental ringworm in cattle. Vet. Rec. **68:** 729—731.

DIFERENCIACION BIOQUIMICA DE ALGUNAS ESPECIES DE ACTINOMYCETES PATOGENOS.

L. F. Bojalil, A. Trujillo & J. Cerbon

Unidad de Patología, Escuela de Medicina, U.N.A.M. Hospital General, México 7, D.F. México.

(6.IV.1959)

La identificación específica en los actinomycetes está basada sobre diferencias en grado de propiedades culturales, tintoriales, morfológicas o fisiológicas, estas características no siempre han sido aplicadas con propiedad a juzgar por el gran número de esquemas de clasificación propuestos para estos microorganismos. (Ørskov, 1923; Jensen, 1931; Duche, 1934; Erikson, 1935; Baldacci 1939).

En el Manual de Bergey (1957), se aceptan como válidas un gran número de especies e incluye algunas que sólo se diferencian en el color del estroma o en la morfología colonial, características consideradas como variables por algunos autores (Jones, 1949; Erikson, 1953). En realidad no existe criterio definido para la identificación de especies aunque trabajos recientes han resaltado la importancia de algunas pruebas bioquímicas para la diferenciación genérica y específica de este grupo (González Ochoa, 1945; McClung, 1954; González Ochoa & Sandoval, 1955; Mariat & Lavalle, 1955; Gordon & Smith, 1955; González Ochoa & Sandoval, 1956; Gordon & Mihm, 1957; Schneidau & Shaffer, 1957; Mariat, 1957; Mariat, 1958).

En el presente trabajo se hizo el estudio de algunas propiedades fisiológicas de actinomycetes patógenos y saprofitos con referencia especial a *N. brasiliensis* y *N. asteroides*; se discute además la aplicación de los datos obtenidos a la clasificación de dichos microorganismos y a la validez que como especie le corresponde a *N. brasiliensis*, organismo de gran-importancia médica en nuestro medio.

Material y Método

Cepas. Los organismos de prueba se recibieron de diversas instituciones con la siguiente denominación:

N. asteroides	20	ISET
N. asteroides	50	,,
N. asteroides	3399	,,
N. asteroides	1160	,,
N. asteroides	151	,,
N. asteroides	652	,,
N. asteroides	1109	,,
N. asteroides	383	,,
N. asteroides	9956A	,,
N. asteroides	19	,,
N. asteroides	1602	,,
N. asteroides	549	CDC
N. asteroides	347	,,
N. asteroides	129	M.C.
N. asteroides	130	M.C.
N. asteroides	131	M.C.
N. asteroides	132	M.C.
N. asteroides	R-217	UPHG
N. asteroides	C-98	,,
N. brasiliensis	1913	ISET
N. brasiliensis	479	,,
N. brasiliensis	477	,,
N. brasiliensis	416	,,
N. brasiliensis	1005	,,
N. brasiliensis	22	UPHG
N. brasiliensis	23	,,
N. brasiliensis	24	,,
N. brasiliensis	36	,,
N. brasiliensis	37	,,
N. brasiliensis	38	,,
N. brasiliensis	39	,,
N. brasiliensis	40	,,
N. brasiliensis	41	,,
N. brasiliensis	54	,,
N. corallina	4273	ATCC
N. corneus	616	ISET
N. leishmanii	4064	IHM
N. melanosporus	145	IHM
N. pretoriana	2391	IMRU
N. asteroides	694	IHM
N. asteroides	789	,,
N. asteroides	1406	,,
N. asteroides	1457	,,
N. asteroides	583	IMRU
N. asteroides	427	,,
N. asteroides	W3661	,,
N. asteroides	529	,,
N. asteroides	420-1	,,
N. asteroides	420-2	,,
N. asteroides	1669	CDC
N. brasiliensis	55	UPHG
N. brasiliensis	56	,,
N. brasiliensis	57	,,
N. brasiliensis	852	,,
N. brasiliensis	766	,,
N. brasiliensis	180	,,
S. madurae 1)	1004	ISET
S. madurae 1)	1110	IOCB
S. madurae 1)	542	ISPB
S. pelletieri 1)	272	IHM
S. somaliensis 1)	1064	IHM
S. paraguayensis 1)	951	IHM
Streptomyces sp.	643	CDC
Streptomyces sp.	804-2	CDC
Streptomyces sp.	58	UPHG
N. pasteuroides	35	ISET
N. convoluta	98	,,
N. rhodnii	4063	IHM
N. transvalensis	2392	IMRU
N. rhodnii	547	FMUSP
N. phenotolerans	1139	UC.
N. polychromogenes	3409	ATCC
N. minima	8674	ATCC
Nocardia sp.	52	UPHG
N. globerula	9356	ATCC
N. gypsoides	9911	NHI(ISET)
N. blackwellii	6846	ATCC

Los autores agradecen a los siguientes investigadores, el habernos proporcionado cepas para este estudio: Dr. A. González Ochoa, ISET, México, D.F.; Dr. J. E. Mackinnon, IH, Montevideo, Uruguay; Dr. R. E. Gordon, Ruttgers University, U. S. A.; Dr. L. Ajello y Dr. L. Georg C. D. C. Atlanta, Ga. U. S. A.; Dr. J. Ulrich, Mayo Clinic, U. S. A.; Dr. C. da Silva Lacaz, Universidad de São Paulo, Brasil y Dr. C. Casas Campillo E.N.C.B., México, D.F.

Significado de las abreviaturas

ISET.	Instituto de Salubridad y Enfermedades Tropicales, México
IHM.	Instituto de Higiene de Montevideo, Uruguay
IMRU.	Institute of Microbiology, Rutgers University, U.S.A.
CDC.	Communicable Disease Center, Atlanta Ga. U.S.A.
M.C.	Mayo Clinic, Rochester Min. U.S.A.
UPHG.	Unidad de Patología, Hospital General, México.
IOCB.	Instituto Oswaldo Cruz, Rio de Janeiro, Brasil.

---

1) Estas especies fueron recibidas y consideradas como *Streptomyces* de acuerdo con lo establecido por González Ochoa & Sandoval (1956) y Mackinnon & Artagaveytia (1956).

ISPB.	Instituto de Saude Publica de Bahía, Brasil.
FMUSP.	Faculdade de Medicina Da Universidade de Sao Paulo, Brasil.
U.C.	Universidad de Colombia Bogotá.
ATCC.	American Type Culture Collection, U.S.A.

Todas las cepas se conservaron en medio de Sabouraud.

Morfología microscópica.

Se utilizó la técnica de microcultivo descrita por Ridell (1950), empleando como medio de cultivo — agar de Bennett y agar extracto de suelo (Gordon & Smith, 1955). Las lecturas se hicieron en preparaciones teñidas por la técnica de Ziehl-Neelsen a los 3, 7 y 14 días de incubación a 28° C; se anotó las características de ramificación, fragmentación y ácido resistencia.

Hidrólisis de caseína.

Se sembraron en estría series por duplicado de cajas de petri conteniendo 20 ml del medio siguiente: — 10.5 g de leche descremada en polvo disuelta en 100 ml de agua a la que se añadió un volumen igual de agar al 2 %, los ingredientes se esterilizaron por separado 15 Lbs durante 20 min, se enfriaron a 47° C, se mezcló y envasó en cajas de petri estériles. La prueba es positiva cuando se observa un halo claro alrededor del cultivo entre 7 y 14 días.

Coagulación de la leche.

Se utilizó el método de Waksman con ligeras modificaciones (González Ochoa, 1945) que consiste en adicionar 3 ml de un cultivo de 1 mes en leche, a un tubo conteniendo 10 ml de leche tornasolada estéril sin pasteurizar; 3 días después se hace la lectura.

Utilización de tirosina.

Se sembraron duplicados de placas conteniendo el siguiente medio de cultivo; peptona 5 g, extracto de carne 3 g; agar 15 g; agua destilada en la que se suspenden 5 g de tirosina 100 ml; se ajusta el pH a 7.0. La desaparición de los cristales de tirosina de las zonas vecinas al crecimiento, indica su utilización; se hicieron lecturas a los 14 y 21 días.

Hidrólisis de gelatina.

Se utilizaron placas conteniendo el siguiente medio de cultivo: a la base descrita para la utilización de tirosina se le adicionaron 4 g de gelatina en sustitución de la tirosina. La utilización se puso de manifiesto a — los 5 ó 10 días por la adición de un agente precipitante de — la gelatina compuesto por 11 g de cloruro mercúrico disueltos en 100 ml de HCl al 28.5 %; la presencia de una zona libre de precipitado alrededor del crecimiento nos indicó la hidrólisis del sustrato.

Comparativamente se verificó la prueba de la licuación de gelatina en tubo por el método utilizado por GONZÁLEZ OCHOA (1945), que consiste en adicionar 3 ml de un cultivo en leche de 4 semanas de incubación a un tubo conteniendo 10 ml de gelatina al 15 %, la incubación se hizo a 37° C y las lecturas a los 3 y 5 días, después de haber puesto los tubos a 4° C durante 30 minutos.

Producción de ácido a partir de diferentes carbohidratos.

Medio basal: $(NH_4)_2$ HPO_4, 1 g; KCl, 0.2; MgSO4, 0.2 g; agua destilada, 1000 ml; bromocresol purpura al 0.04 % en solución acuosa, 15 ml; ajustar el pH a 7.0; agar, 15 g; esterilizar a 15 lbs durante 15 min, adicionar el carbohidrato que se quiere probar en cantidad suficiente para obtener una concentración final de 1.0 %. Se utilizaron los siguientes azucares: lactosa, xilosa, manosa, maltosa, galactosa, inositol, glucosa, dulcitol, fructosa, ramnosa, rafinosa, sacarosa, melibiosa, sorbitol, manitol y arabinosa. Toda las observaciones se hicieron 7 y 28 — días de incubación a 28° C. Todas las cepas se sembraron en caldo albúmina de huevo para comprobar su pureza.

RESULTADOS

Los resultados están expresados en las tablas en forma de cociente: el numerador representa el número de cepas que — dieron la prueba positiva y el denominador el número de cepas estudiadas.

Morfología microscópica.

En el cuadro I se anotan las características de micelio aéreo ramificación, fragmentación y ácido-resistencia. Se observa que

TABLA I.

Caracteristicas Microscopicas de los Generos Nocardia y Streptomyces.

Caracteristicas	*Nocardia asteroides*	*Nocardia brasiliensis*	*Streptomyces*	*Otras Nocardias*
Presencia de micelio aéreo	11/30	16/21	3/9	5/17
Ramificación	30/30	21/21	9/9	17/17
Fragmentación	30/30	21/21	0/9	17/17
Acido Resistencia	27/30	19/21	0/9	15/17

El numerador indica el número de cepas que presentaron las características anotadas.

El denominador indica el número total de cepas estudiadas.

la presencia de micelio aéreo puede no ocurrir incluso dentro de las especies del género — *Streptomyces*, como en el caso de las especies patógenas *S. pelletieri*, *S. madurae* y *S. somaliensis*; la fragmentación y ácido resistencia sólo se presentan en las especies del género *Nocardia*, aunque la última propiedad no siempre fué constante.

En la tabla II, se anotan los resultados de las pruebas fisiológicas para *Nocardia* y *Streptomyces*.

Fueron las siguientes propiedades las de mayor valor para la caracterización de los grupos: *N. asteroides*, no descompuso la caseína ni disolvió los cristales de tirosina; sólo hidrolizó la gelatina en algunas ocasiones.

TABLA II.

Características fisiológicas de los géneros Nocardia y Streptomyces

Pruebas	*Nocardia asteroides*	*Nocardia brasiliensis*	*Streptomyces*
Descomposición de:			
Caseína	0/30	21/21	9/9
Tirosina	0/30	19/21	9/9
Hidrólisis de gelatina	3/30	21/21	9/9
Coagulación de leche	2/30	18/21	4/9
Licuación de gelatina	2/30	18/21	7/9

Producción de ácido a partir de:

Lactosa	5/30	1/21	4/9
Xilosa	6/30	1/21	7/9
Manosa	11/30	5/21	8/9
Maltosa	7/30	1/21	2/9
Galactosa	9/30	21/21	5/9
Inositol	9/30	17/21	5/9
Glucosa	30/30	21/21	9/9
Dulcitol	0/30	0/21	0/9
Fructosa	15/30	19/21	7/9
Ramnosa	6/30	0/21	5/9
Rafinosa	0/30	0/21	3/9
Sacarosa	2/30	0/21	5/9
Melibiosa	0/30	0/21	5/9
Sorbitol	2/30	0/21	5/9
Manitol	6/30	8/21	3/9
Arabinosa	2/30	3/21	7/9

El numerador indica el número de cepas que dieron la prueba positiva.
El denominador el número de cepas estudiadas.

TABLA III.

Propiedades fisiológicas de diversas especies del género Nocardia.

Pruebas	*N. leishmanii*	*N. pretoriana*	*N. rhodnii* 4063	*N. gypsoides*	*N. transvalensis*	*N. convoluta*	*N. polychromogenes*	*N. globerula*	*N. blackwellii*	*N. corallina*	*N. minima*	*N. pasteuroides*	*N. phenotolerans*	*N. corneus*	*N. melanosporus*	*N. rhodnii* 547	*Nocardia* sp. 52
Descomposición de caseína	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+
Coagulación de leche	+	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	+
Utilización de Tirosina	—	+	+	—	—	—	—	—	—	+	—	—	—	—	—	+	+
Hidrolisis de gelatina	—	+	—	—	—	—	—	—	+	—	—	—	—	—	—	—	+
Licuación de gelatina	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+
Producción de ácido a partir de:																	
Lactosa	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+
Xilosa	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Manosa	—	+	+	—	—	—	—	+	—	—	—	—	—	—	+	+	+
Maltosa	—	—	+	—	—	—	—	—	—	—	—	—	+	—	—	—	+
Galactosa	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—
Inositol	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+
Glucosa	+	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+
Dulcitol	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Fructosa	—	+	+	—	+	—	—	+	+	+	—	—	+	—	+	+	+
Ramnosa	—	—	—	—	—	—	+	—	—	—	+	—	—	—	—	—	[illegible]
Rafinosa	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sacarosa	—	—	+	—	—	—	+	—	—	—	+	—	—	—		—	—
Melibiosa	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Sorbitol	—	—	+	—	—	—	—	+	—	+	—	—	—	—	—	—	—
Manitol	—	+	+	+	+	+	—	+	—	+	—	—	—	—	—	—	—
Arabinosa	—	—	+	—	—	—	—	—	—	—		—	—	—	—	—	—

N. brasiliensis y *Streptomyces*, descompusieron la caseína, disolvieron los cristales de tirosina e hidrolizaron la gelatina en forma constante. La capacidad de todos estos microorganismos para producir ácido a partir de azúcares es variable.

En la tabla III, se anotan las características fisiológicas de algunas nocardias recibidas con diferentes denominaciones. Nótese que la mayoría de ellas muestran una actividad similar a la observada para *N. asteroides*; las excepciones fueron las siguientes: *N. pretoriana*, *N. rohdnii* y *N. corallina* que tuvieron actividad sobre tirosina.

Nocardia sp. 52, presentó las mismas características que anotamos para *N. brasiliensis*.

DISCUSIÓN

De los resultados obtenidos, puede apreciarse que la fragmentación, definida como la segmentación de la hifa en elementos cocoides y bacilares, es constante para todas las especies del género *Nocardia*; esta propiedad no se observó en ninguna de las especies de *Streptomyces*. Otra característica importante del género *Nocardia* es su ácido resistencia, aunque en ocasiones variable cuando la tinción se hace a partir de frotis de cultivos, es sumamente constante si se investiga a partir de material patológico (infección experimental). La fragmentación es de mucha utilidad en la diferenciación de *Nocardia* y — *Streptomyces*, fundamentalmente de *S. pelletieri*, *S. madurae* y *S. somaliensis*, en los que la esporulación es sumamente difícil de demostrar.

Por las características bioquímicas, observamos que existen dos grandes grupos dentro del género *Nocardia*:

Grupo 1. Microorganismos que utilizan la caseína y tirosina, hidrolizan la gelatina, coagulan la leche y producen ácido a partir de inositol, galactosa, glucosa y fructosa con algunas excepciones; no producen ácido a partir de dulcitol, ramnosa, rafinosa, sacarosa, melibiosa y sorbitol; en este grupo se incluyen solamente las cepas recibidas como *Nocardia brasiliensis*, a la cual correspondió también *Nocardia* sp. 52.

Grupo 2. Microorganismos de baja actividad que no descomponen la caseína ni la tirosina, excepcionalmente hidrolizan — la gelatina y coagulan la leche, producen ácido a partir de — glucosa. No producen ácido de dulcitol, rafinosa o melibiosa; y sólo ocasionalmente lo producen a partir de otros azúcares.

En este grupo de baja actividad fisiológica incluimos — todas las cepas recibidas como *N. asteroides* y otras nocardias como *N. leishmanii*, *N. gypsoides*, *N. transvalensis*, *N. convoluta*, *N. polychromogenes*, *N. globerula*, *N. blackwellii*, *N. minima*, *N. pasteuroides*, *N. phenotolerans*, *N. corneus* y *N. melanosporus*, que presentaron actividad similar a *N. asteroides*; de acuerdo con los resultados obtenidos, estas últimas especies deben ser consideradas como *N. asteroides*. Por otro lado *N. pretoriana*, *N. Rhodnii* y *N. corallina*, tienen en común con *N. asteroides* la incapacidad de utilizar la caseina, coagular la leche y una baja actividad en la producción de ácido a partir de azúcares; sin embargo, estas cepas descomponen la tirosina, característica que no presentaron ninguna de las cepas recibidas como *N. asteroides*, por lo que consideramos necesario realizar estudios adicionales tales como patogenicidad, — composición química de pared celular y propiedades serológicas para decidir la posición taxonómica de estas "especies".

Las propiedades bioquímicas de *Streptomyces* fueron similares a las de *N. brasiliensis* en lo que se refiere e descomposición de caseína, tirosina, hidrólisis de gelatina y coagulación de la leche; *Streptomyces* mostró mayor capacidad de — producción de ácido a partir de azúcares; sin embargo, esta — capacidad no es homogénea y por lo tanto la separación del género *Streptomyces* de la especie *N. brasiliensis* no es posible basarla en estas pruebas. La ausencia de fragmentación y ácido resistencia son, además de la producción de esporas, las únicas características diferenciales observadas.

Otros autores (MARIAT, 1958) encuentran que la utilización de parafina por *Nocardia* y no por *Streptomyces* puede servir para hacer la separación de estos géneros; sin embargo, — existen comunicaciones que muestran que *S. madurae* es capaz de utilizar esta substancia mientras que *N. caprae* y *N. gardneri* no la utilizan (SCHNEIDAU & SHAFFER, 1957).

La diferente composición de pared celular observada para *Nocardia* y *Streptomyces* por ROMANO & SOHLER (1956) y CUMMINS & HARRIS, (1958), quizá podría servir para una mejor diferenciación genérica.

RESUMEN

Se realizó un estudio de diversas propiedades de *N. asteroides*, *N. brasiliensis* y algunos *Streptomyces* patógenos.

Se investigaron los siguientes puntos: a) morfología microscópica, b) descomposición de diversas substancias tales como caseína y tirosina, c) hidrólisis de gelatina y — coagulación de leche, d) producción de ácido a partir de diferentes carbohidratos.

De los resultados se deduce que existen dos grandes grupos dentro el género *Nocardia*:

Grupo 1. Caracterizado por utilizar la caseína y tirosina, hidrolizar la gelatina, coagular la leche y producir generalmente ácido a partir de inositol, galactosa, glucosa y fructosa; a este grupo pertenece exclusivamente *Nocardia brasiliensis*.

Grupo 2. Microorganismos caracterizados por su baja actividad, no descomponen la caseína ni la tirosina, excepcionalmente hidrolizan la gelatina y coagulan la leche, producen ácido a partir de glucosa. En este grupo incluímos todas las cepas recibidas como *N. asteroides* y otras tales como — *N. leishmanii*, *N. gypsoides*, *N. transvalensis*, *N. convoluta*, *N. polychromogenes*, *N. globerula*, *N. blackwellii*, *N. minima*, *N. pasteuroides*, *N. phenotolerans*, *N. corneus* y *N. melanosporus*.

Por otra parte *N. pretoriana*, *N. rhodnii* y *N. corallina*, tienen en común con *N. asteroides* la incapacidad de utilizar la caseína y coagular la leche pero estas cepas descomponen la tirosina, característica que no presentó ninguna de las cepas clasificadas como *N. asteroides*, por lo que se considera necesario hacer estudios adicionales para decidir la posición taxonómica de estas "especies".

Las propiedades bioquímicas de *Streptomyces* fueron similares a las de *N. brasiliensis* por lo que la separación del género *Streptomyces* de *N. brasiliensis* no es posible basarla en estas pruebas, siendo la ausencia de fragmentación y ácido resistencia, además de la producción de esporas, las únicas características diferenciales observables.

SUMMARY

In this paper a study is presented of the various properties of *N. asteroides*, *N. brasiliensis* and some pathogenic *Streptomyces*.

The following aspects were studied: a) microscopic morphology, b) descomposition of several substances such as casein and tyrosine, c) gelatin hydrolysis and milk coagulation, d) acid production using different carbohydrate.

From the results obtained it is deduced that two main groups exist within the genus *Nocardia*:

Group 1. Characterized by their ability to utilize casein and tyrosine, hydrolyse gelatin, coagulate milk and usually producing acid from inositol, galactose, glucose and fructose: only *Nocardia brasiliensis* belongs to this group.

Group II. Microorganisms characterized by their low activity, inability to decompose casein and tyrosine, exceptionally they hydrolyse gelatin or coagulate milk. In this group we include all the strains received as *N. asteroides* and others such as *N. leishmanii*, *N. gypsoides*, *N. transvalensis*, *N. convoluta*, *N. polychromogenes*, *N. globerula*, *N. blackwellii*, *N. minima*, *N. pasteuroides*, *N. phenotolerans*, *N. corneus* and *N. melanosporus*.

On the other hand *N. pretoriana*, *N. rhodnii* and *N. corallina*, have in common with *N. asteroides* the inability to utilize casein or coagulate milk, however, these strains decompose tyrosine, a characteristic which is not present in any other strains classified as *N. asteroides*, therefore it is necessary to carry out additional studies so that the taxonomic position of these "species" may be decided.

The biochemical properties of *Streptomyces* were similar to those of *N. brasiliensis*, therefore it is not possible through these methods, to make the separation of the genus *Streptomyces* from *N. brasiliensis*, the absence of fragmentation, the acid fastness and production of spores are the only differential characteristics that could be observed.

REFERENCIAS

BALDACCI, E. (1939) Introduzione allo studio degli attinomiceti, Mycopath., **2,** 84—106.

BREED, R. S., MURRAY, E. G. D. & SMITH, N. R. (1957) Bergey's Manual of Determinative Bacteriology. 7 ed. The Williams & Wilkins Co. Baltimore.

CUMMINS, C. S. & HARRIS, H. (1958) Studies on the cell-wall composition and taxonomy of Actinomycetales and related groups. J. gen. Microbiol. **18,** 173—189.

DUCHE, J. (1934) Les Actinomyces du Groupe Albus, Encyclopédie Mycologique. VI, Paul Lechevalier & Fils, Paris. (Citado por WAKSMAN, 1950).

ERIKSON, D. (1935) The pathogenic aerobic organisms of the actinomyces group. Med. Res. Council. London. **203,** 5—61.

ERIKSON, D. (1953) Variation of mycelial pattern in sporogenous and asporogenous actinomycetes. Symposium on The Actinomycetales. 6 th. Int. Congr. Microbiol. Roma Italy, p. 102.

GONZÁLEZ OCHOA, A. (1945) Estudio comparativo entre *Actinomyces mexicanus*, *A. brasiliensis* y *A. asteroides*. Rev. Inst. Salub. y Enf. Trop. **6,** 155—162.

GONZÁLEZ OCHOA, A. & SANDOVAL, M. A. (1955) Características de los actinomicetes patógenos más comunes. Rev. Inst. — Salub. y Enf. Trop., **15**, 149—161.

GONZÁLEZ OCHOA, A. & SANDOVAL, M. A. (1956) Revisión determinativa de algunas especies de actinomicetos patógenas — descritas como diferentes. Rev. Inst. Salub. y Enf. — Trop., **16**, 17—25.

GORDON, R. E. & MIHM, J. M. (1957) A comparative study of some strains received as *Nocardia*. J. Bact., **73**, 15—27.

GORDON, R. E. & SMITH, M. N. (1955) Proposed groups of characters for the separation of *Streptomyces* and *Nocardia*. J. Bact. **69**, 147—150.

JENSEN, H. L. (1931) Contribution to our knowledge of the actinomycetales II. — The definition and subdivision of the genus *Actinomyces*, with a preliminary account of Australian soil actinomycetes. Proc. Linn. Soc. N. S. Wales, **56**, 345—370.

JONES, K. L. (1949) Fresh-isolates of actinomycetes in wich the presence of sporogenus aerial mycelium is a fluctuating characteristic. J. Bact., **57**, 141—145.

MCCLUNG, N. M. (1954) The utilization of carbon compounds by *Nocardia* species. J. Bact., **68**, 231—236.

MACKINNON, J. E. & ARTAGAVEYTIA-ALLENDE, R. C. (1956) The main species of pathogenic aerobic actinomycetes causing mycetomas. Trans. Roy. Soc. trop. Med. Hyg., **50**, 31—40.

MARIAT, F. (1957) Sur l'utilization de divérs composés carbonés et azotés par *Streptomyces madurae*, *S. pelletieri* et *S. somaliensis*. C. R. Acad. Sci. Paris. **245**, 593—596.

MARIAT, F. (1958) Physiologie des Actinomycetes aeróbies pathogènes. Recherches sur l'activité proteolytique et sur la nutrition azotée et carbonée de *Nocardia asteroides*, *N. brasiliensis*, *Streptomyces madurae*, *S. pelletieri* et *S. somaliensis*. Mycopath. et Mycol. Appl. **9**, 111—149.

MARIAT, F. & LAVALLE, P. (1955) Sur l'utilization de divers composés carbonés et azotés par *N. asteroides* et *N. brasiliensis*. C. R. Acad. Sci. Paris, **240**, 255—257.

ORSKOV, J. (1923) Investigations into the morphology of the ráy-fungi. Copenhagen. (Citado por ERIKSON, 1935).

RIDELL, W. R. (1950) Permanent stained mycological preparations obtained by slide culture. Mycologia, **42**, 265—270.

ROMANO, A. H. & SOHLER, A. (1950) Biochemistry of the actinomycetales II. A comparison of the cell-wall composition — of species of the genera *Streptomyces* and *Nocardia*. J. Bact., **72**, 865—868.

SCHNEIDAU, J. D. & SHAFFER, F. M. (1957) Studies of *Nocardia* and other Actinomycetales. Amer. Rev. Tuberc., **76**, 770—788.

WAKSMAN, S. A. (1940) On the classification of actinomycetes. J. Bact., **39**, 549-558.

WAKSMAN, S. A. & HENRICI, A. T. (1943) The nomenclature and classification of the actinomycetes. J. Bact. **46** 337—341.

DEVELOPMENTAL MORPHOLOGY IN THE GENUS NOCARDIA

by

Barbara Burns Young & Margaret Hotchkiss

Department of Bacteriology, University of Kentucky, Lexington, Kentucky

(8.VII.1959)

Few pictorial records exist in the literature that can aid in the identification of the aerobic Actinomycetes. The pleomorphism that distinguishes this group from other bacteria was recognized by Klieneberger-Nobel (1947) and by Erikson (1948, 1949b). Erikson (1949a) states that Novak & Henrici (1933) described the pleomorphism of an 'undoubted member of the genus *Nocardia.*' All workers agree that certain strains give rise to spherical cells which have sometimes been labeled 'coccoid bodies' and sometimes 'spores'. The origin and function of these bodies is obscure. For our studies we borrowed the microculture technique of Mc Clung (1949, 1950, 1954) who had followed the growth of many isolates of *Nocardia* during a 72-hour period.

At this time we had available three isolates, representing the three species groups that are common in producing nocardiosis in Mexico. (Gonzales-Ochoa & Angeles-Sandoval, 1955) The cultures of *N. asteroides* and *N. madurae* were obtained at the *Instituto de Salubridad y de Enfermedades Tropicales* in Mexico City, and the culture of *N. brasiliensis* was isolated by one of us in the same laboratory.

Since the organisms are not fastidious, a simple medium was used to maintain cultures and to prepare microcultures. It consisted of:

Casitone (Difco)	10	g
Glucose	40	g
Magnesium sulfate	0.1	g
Phosphate buffer pH 6.8 . . .	200	ml
Distilled water q.s.	1000	ml

Solid medium was prepared by the addition of 2% agar.

Microcultures were prepared either as hanging drop slides of inoculated liquid medium or by growing the organisms on blocks of agar set on a sterile microscope slide. Over the block of inoculated agar a sterile cover slip was placed. The microcultures were kept in moist chambers, prepared in petri dishes and incubated at 30° C. They were observed daily during a six-week incubation period. A series of cultures was inoculated at the same time, and whenever a change in morphology occurred, the culture was sacrificed, a permanent mount was made and the preparation was photographed.

Excessive manipulation was avoided in order to leave the growth *in situ*. The coverslip, to which the growth adhered, was removed and dried in a desiccator over calcium chloride. After drying, the preparations were fixed with two applications of absolute methyl alcohol. They were stained with HUCKER'S modification of GRAM'S method.

Photographs were taken with an Argus C-3 camera and a 1/3 × photographic attachment and a 10 × ocular with Bausch and Lomb objectives either 10 ×, 44 × or 97 ×.

The inocula were prepared from cultures that had grown for six weeks. It was found that the coccoid bodies from *N. asteroides* could be obtained from the sediment of a tube of liquid culture that had been allowed to stand for six weeks. (Fig. 1) If fragmented hyphae were desired, a portion of the pellicle from a six-week culture of either *N. asteroides* or *N. brasiliensis* was removed and shaken with glass beads. In a similar manner, a sedimented colony from a broth culture of *N. madurae* was removed and fragmented.

The first series of photographs shows the developmental morphology of *N. asteroides* in liquid medium. Figure 1 shows the inoculum of gram positive (very dark) and gram negative (very light) coccoid elements. After three days incubation the coccoid bodies appeared in clumps or pairs (Fig. 2). Some of the coccoid bodies elongated to form curved rods and figure 3 shows a circular structure. This circular structure could have been the result of the elongation of both members of a pair of coccoid bodies or, perhaps the bending of a germination tubule produced from a single coccoid body. After six days incubation thick gram positive hyphae appeared, which radiated from central points of the young colonies and gave the classic picture of a "ray fungus" (Fig. 4). After three weeks incubation these young colonies had taken on a thick matted appearance (Fig. 5) but a few coccoid bodies could be distinguished at the periphery. The periphery (Figs. 6, 7, 8) at a higher magnification showed hyphae with buds, clusters of coccoid bodies appearing along the hyphae, and numerous coccoid bodies appearing singly, in pairs or clumps with no particular relation to the now gram negative filaments. After six weeks the hyphae appeared to have completely disappeared and figure 9 shows the entire field filled with gram positive coccoid bodies in a mass of degenerated hyphae. Thus we were able to demonstrate a complete cycle of growth from the coccoid bodies, to the filamentous form and back to the coccoid bodies.

Figures 10 through 21 show the development of microcultures of the same strain of *N. asteroides* on solid medium. The inoculum consisted of fragmented mycelial threads. After three days incubation there were free gram positive bacillary forms while the mycelial threads showed beading or granulation (Fig. 10). After two weeks incubation the periphery of the microcolonies consisted of long, thin, slightly twisted filaments with very little condensation of protoplasm (Fig. 11). At the end of three weeks incubation these colonies had begun to disintegrate. The center of one colony was photographed at two magnifications to show the presence of coccoid and bacillary forms in the fragmented portions of the mycelium (Figs. 12, 13). The manner in which thick gram positive elements were attached to thin gram negative portions of the mycelial threads is shown in figure 14. In other areas many coccoid bodies were seen along the course of the hyphae and in some portions of the culture the hyphae seemed to adhere to each other as they radiated towards the periphery (Fig. 15). After six weeks incubation the developing morphology was different in the solid medium from that encountered in the cultures in liquid media. The colonies still seemed intact with a mass of thickly matted mycelium at the center, surrounded by a lighter area, and, at the periphery of the colony, thick darker staining hyphae. The dense center was composed of bacillary forms (Fig. 16), while the light portion proved to be made up of delicate beaded, gram negative filaments (Fig. 17) and these merged with ghost filaments and bacillary bodies (Fig 18). At the periphery of the colony there were no longer ghost filaments but thick, gram positive hyphae with short thick branches. Within these branches many developing spherical bodies were observed and occasionally clumps of coccoid bodies were seen (Figs. 19, 20, 21).

The strain of *N. brasiliensis* grew more rapidly than the strain of *N. asteroides*. After two days incubation in liquid cultures the pattern of the mycelial mat had already developed and figure 22 shows large, gram positive, many-branched hyphae. As the cultures grew there were longer secondary branches and condensation of the protoplasm occurred which resulted in variations in the degree of staining. No free coccoid bodies were observed, but figure 23, from a preparation after two weeks incubation, shows a swelling in one of the hyphae. At the end of three weeks incubation, the culture showed many long, heavy, gram positive filaments in a background of ghost filaments (Fig. 24). In other areas the condensation of protoplasm was marked and seemed to occur in the portion of the mycelium where the thick gram positive filaments had been seen in younger cultures (Fig. 25). After six weeks incubation, the center and the periphery of the colonies showed numerous bacillary forms in a background of degenerated mycelium (Figs. 26, 27).

When grown on solid medium the microcultures of *N. brasiliensis* showed a similar pattern of development (Figs. 28 through 33). At the end of four days incubation the mycelium was quite extensive, while the hyphae, radiating from the center of matted threads, appeared as long gram positive, sparsely branched filaments. After one week incubation the filaments showed more branching and some seemed to cohere as they twisted in the culture medium. Rarely a swelling was observed in the wall of a hypha, but no free coccoid bodies were found. Other areas of the same preparation showed the phenomenon of parallelism and there was evidence of condensation of protoplasm and beading. After six weeks incubation, some areas showed masses of gram negative highly twisted hyphae with gram positive granules. This culture on solid medium differs from the companion culture on liquid medium in that the six weeks culture on solid medium was composed of granules in degenerate hyphae while in liquid medium the residual form was bacillary.

In a similar manner the growth of a strain of *N. madurae* was studied in liquid and solid medium. The series of observations seen in liquid medium are recorded in figures 34 through 41.

At the end of one week this culture seemed quite homogeneous when examined grossly and this was due to numerous colonies, composed of long gently waving filaments with thickly matted centers. At a magnification of 440 × the colonies resemble a "medusa head", and at a magnification of 970 × the coherence of the mycelial threads can be seen (Figs. 34, 35). In some areas hundreds of hyphae seemed to be involved. After two weeks incubation the coherence was not so noticeable, although there were some areas which showed parallel mycelial threads following a common path. The colonies failed to present the classic picture of the "ray fungus". The hyphae themselves retained an even gram positive stain and there appeared to be no condensation of the protoplasm (Figs. 36, 37). However, after three weeks incubation the hyphae were less uniform in diameter, and they stained less evenly, and it was at this age that coccoid bodies, forming terminally or within the hyphae, were first detected. Some of the coccoid bodies appeared to germinate while still attached to the mother hypha. This may be one explanation for the absence of free coccoid bodies, even though many attached coccoid forms were visible. (Figs. 38, 39).

After six weeks incubation many fragments of hyphae were seen. These fragments were not like the bacillary forms seen in the other two test organisms. They were longer, thinner, and less uniform in diameter. Terminally developing coccoid bodies were seen and thickening of the hyphae, giving the appearance of the formation of intercalary elements, was noted. However no free coccoid bodies were seen in the preparation. (Fig. 40, 41).

The growth of *N. madurae* on solid medium is shown in figures 42 through 45. The colonies were gram positive and at a magnification of 100 × the "medusa head" appearance was lacking. When examined at a magnification of 970 × the hyphae at the edges of the colonies showed some coherence. (Fig. 42) After two weeks incubation, a matted mycelium consisting of hyphae of uniform diameter

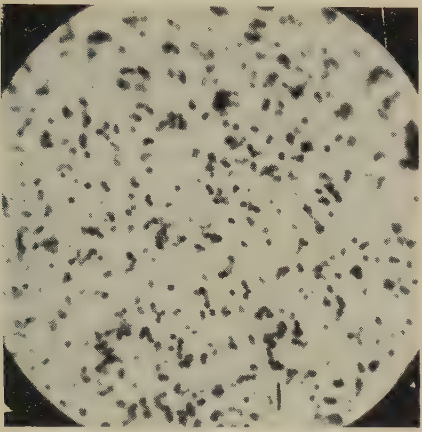


Fig. 1 *N. asteroides*. Microculture before incubation. Liquid medium. 97 × objective, 10 × ocular

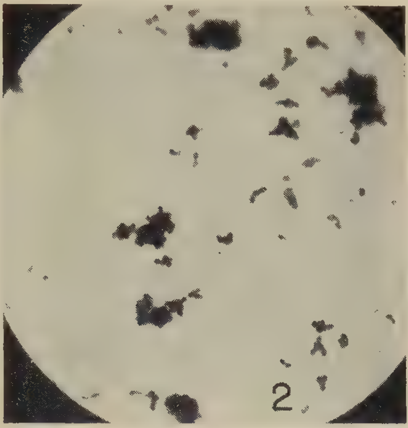


Fig. 2 *N. asteroides*. Three days incubation. 97 × objective, 10 × ocular

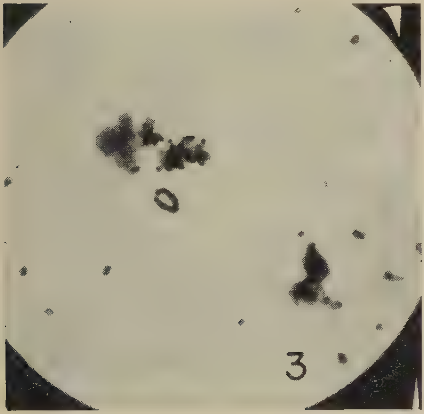


Fig. 3 *N. asteroides*. Three days incubation. 97 × objective, 10 × ocular

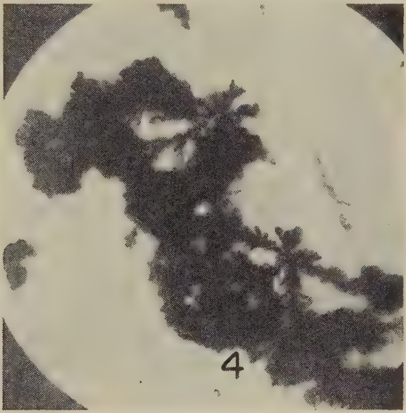


Fig. 4 *N. asteroides*. Six days incubation. 97 × objective, 10 × ocular

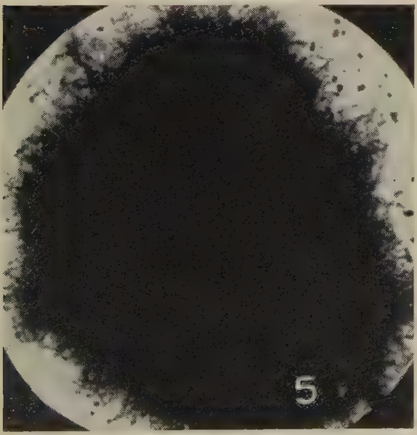


Fig. 5 *N. asteroides*. Six days incubation. 44 × objective, 10 × ocular

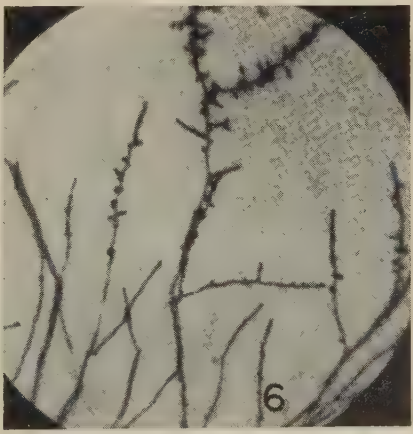


Fig. 6 *N. asteroides*. Three weeks incubation. 97 × objective, 10 × ocular

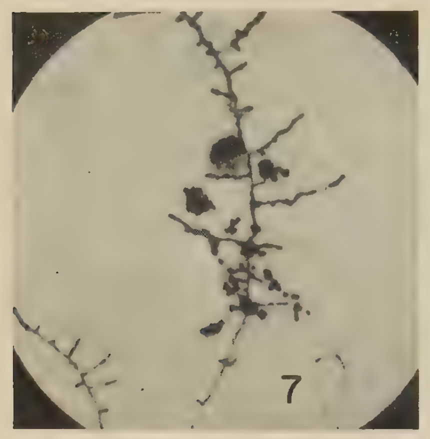


Fig. 7 *N. asteroides*. Three weeks incubation. 97 × objective, 10 × ocular

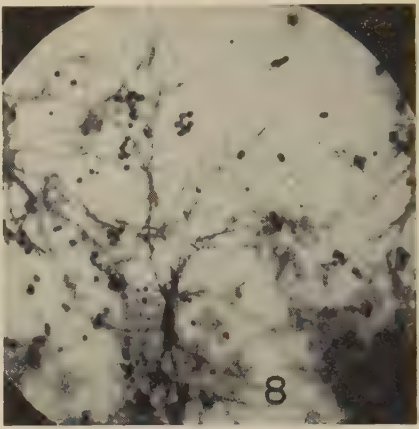


Fig. 8 *N. asteroides*. Three weeks incubation. 97 × objective, 10 × ocular

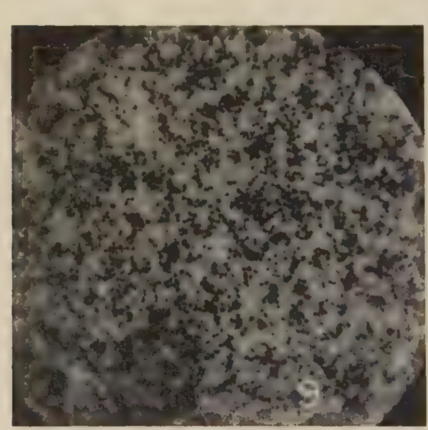


Fig. 9 *N. asteroides*. Six weeks incubation. 97 × objective, 10 × ocular

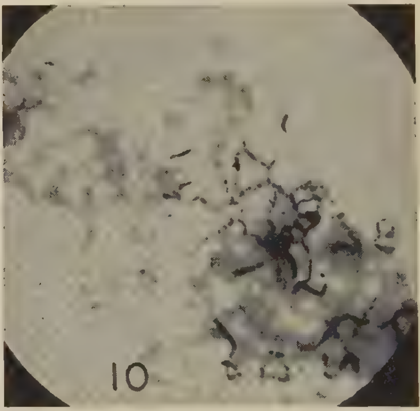


Fig. 10 *N. asteroides*. Microculture inoculated with mycelial fragments. Solid medium. Three days incubation. 97 × objective, 10 × ocular.

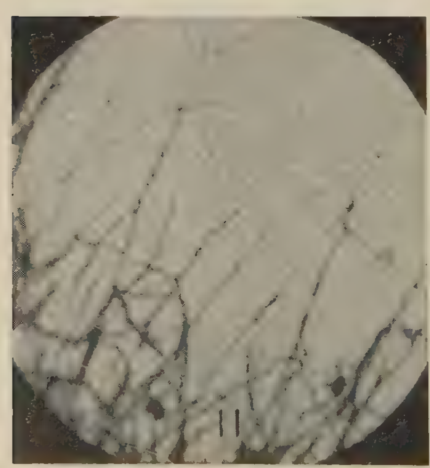


Fig. 11 *N. asteroides*. Two weeks incubation. 97 × objective, 10 × ocular

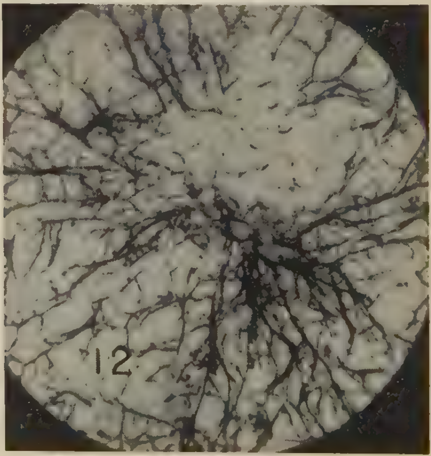


Fig. 12 *N. asteroides*. Three weeks incubation. 44 × objective, 10 × ocular

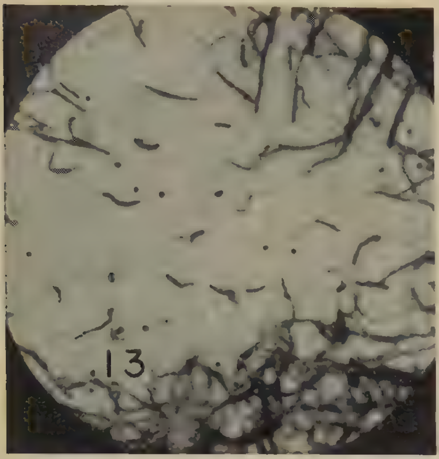


Fig. 13 *N. asteroides.* Three weeks incubation. 97 × objective, 10 × ocular

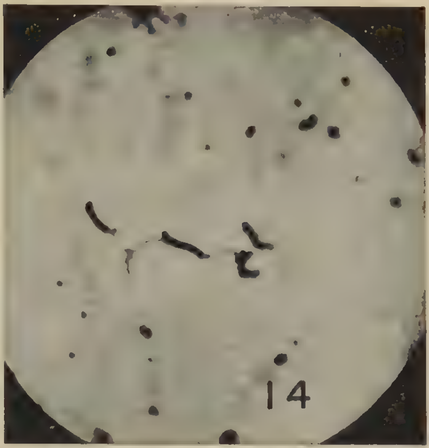


Fig. 14 *N. asteroides.* Three weeks incubation. 97 × objective, 10 × ocular

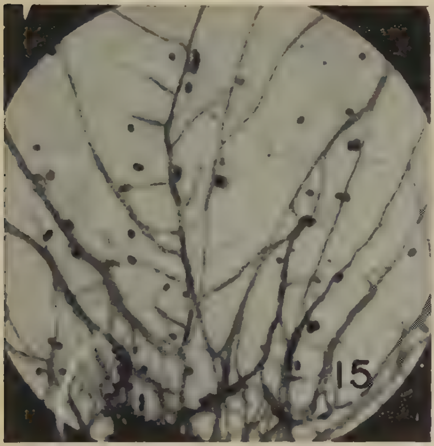


Fig. 15 *N. asteroides.* Three weeks incubation. 97 × objective, 10 × ocular

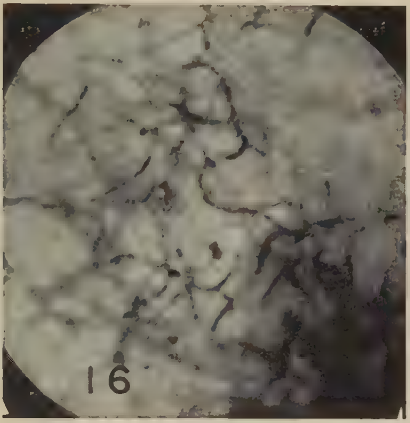


Fig. 16 *N. asteroides.* Six weeks incubation. 97 × objective, 10 × ocular

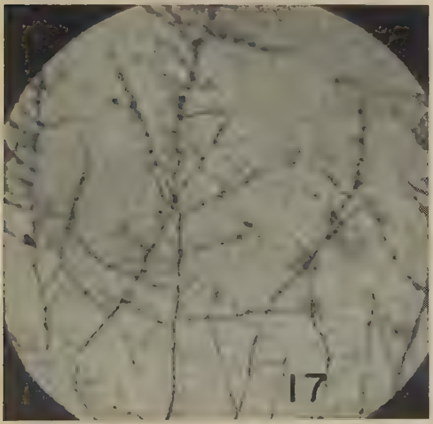


Fig. 17 *N. asteroides.* Six weeks incubation. 97 × objective, 10 × ocular

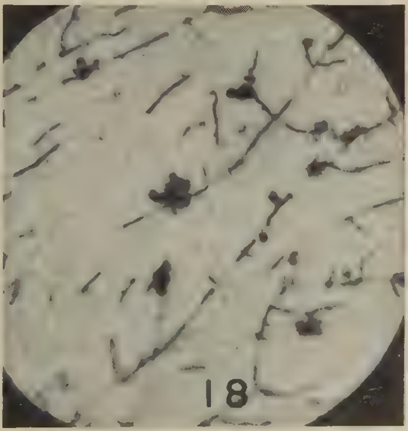


Fig. 18 *N. asteroides.* Six weeks incubation. 97 × objective, 10 × ocular

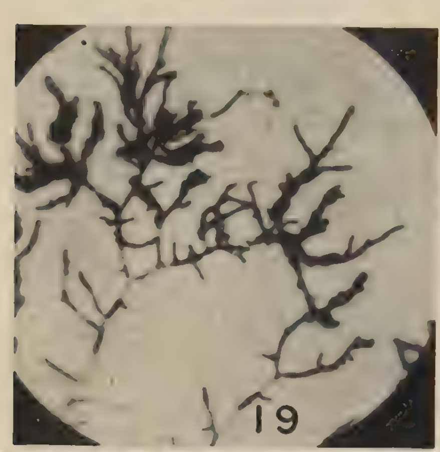


Fig. 19 *N. asteroides*. Six weeks incubation.
97 × objective, 10 × ocular

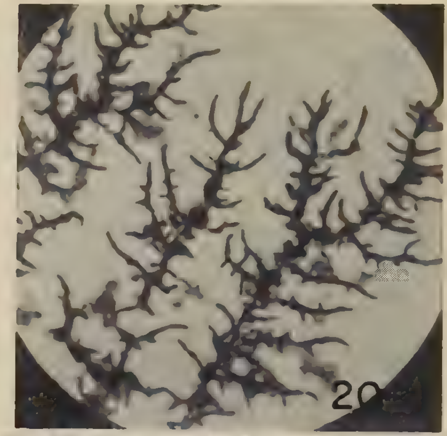


Fig. 20 *N. asteroides*. Six weeks incubation
97 × objective, 10 × ocular

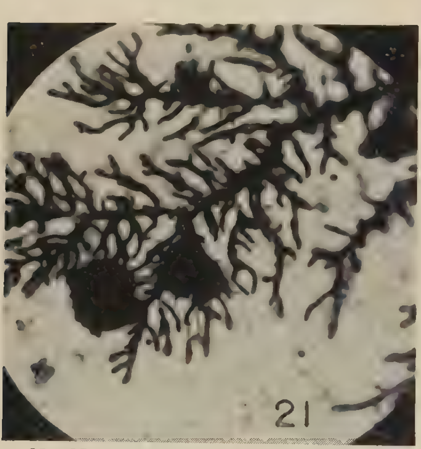


Fig. 21 *N. asteroides*. Six weeks incubation.
97 × objective, 10 × ocular

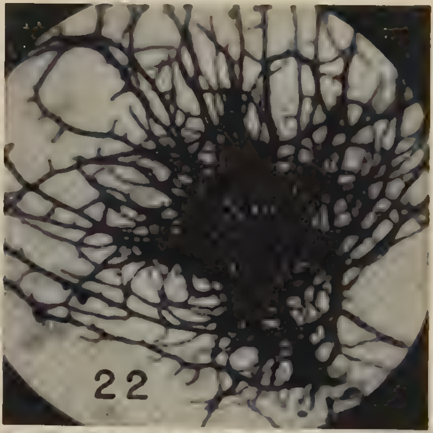


Fig. 22 *N. brasiliensis*. Two days incubation.
Liquid medium 97 × objective, 10 × ocular

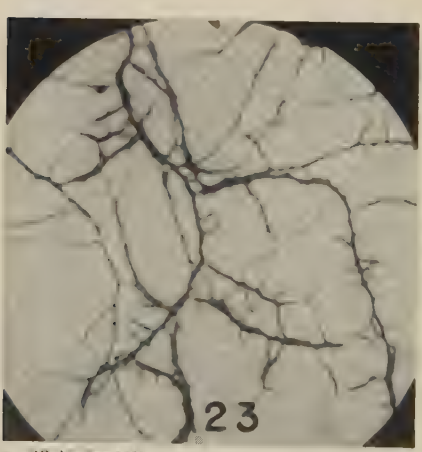


Fig. 23 *N. brasiliensis*. Two weeks incubation.
97 × objective, 10 × ocular

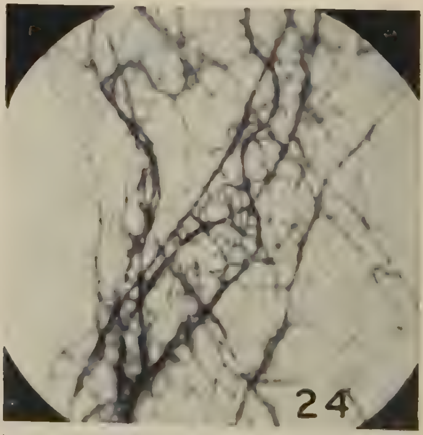


Fig. 24 *N. brasiliensis*. Three weeks incubation.
97 × objective, 10 × ocular

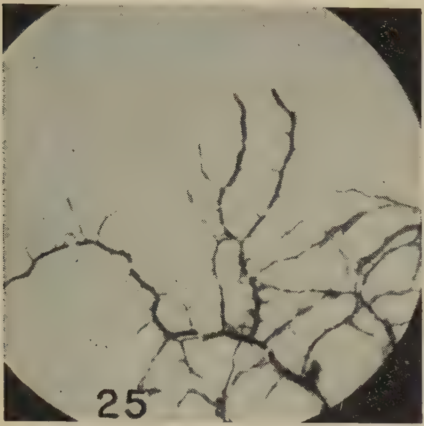


Fig. 25 *N. brasiliensis*. Three weeks incubation. 97 × objective, 10 × ocular

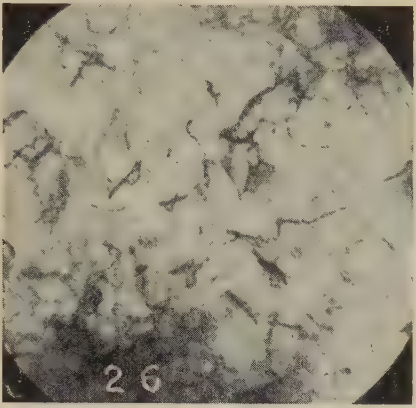


Fig. 26 *N. brasiliensis*. Six weeks incubation. 97 × objective, 10 × ocular

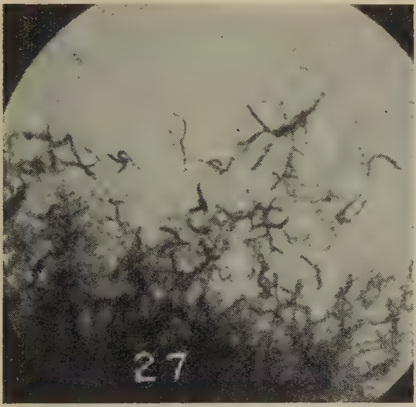


Fig. 27 *N. brasiliensis*. Six weeks incubation. 97 × objective, 10 × ocular

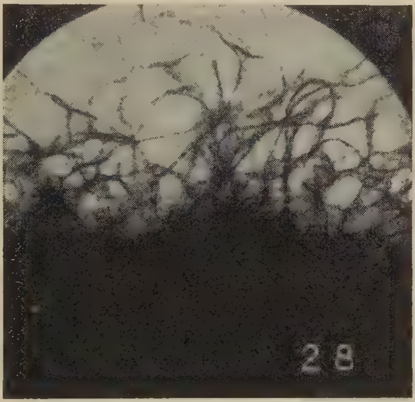


Fig. 28 *N. brasiliensis*. *B* Microculture on solid medium. Four days incubation. 97 × objective, 10 × ocular

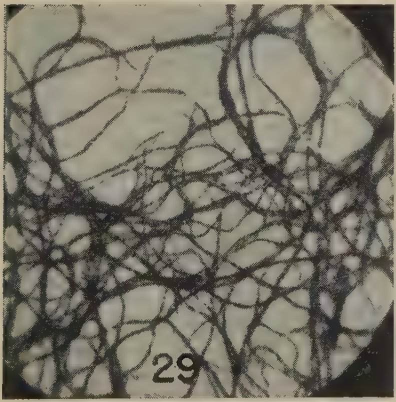


Fig. 29 *N. brasiliensis*. One week incubation. 97 × objective, 10 × ocular

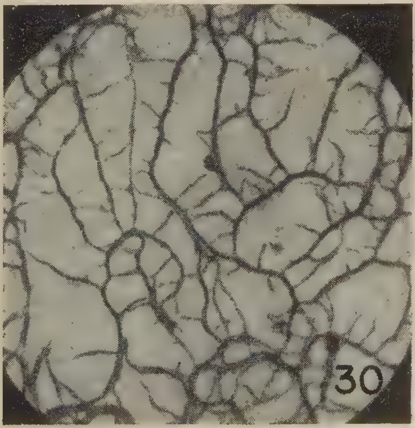


Fig. 30 *N. brasiliensis*. One week in cubation. 97 × objective, 10 × ocular

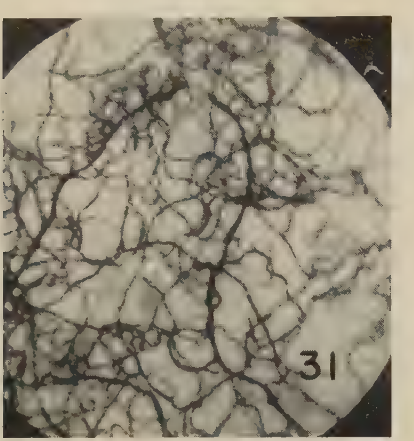


Fig. 31 *N. brasiliensis*. Three weeks incubation. 97 × objective, 10 × ocular

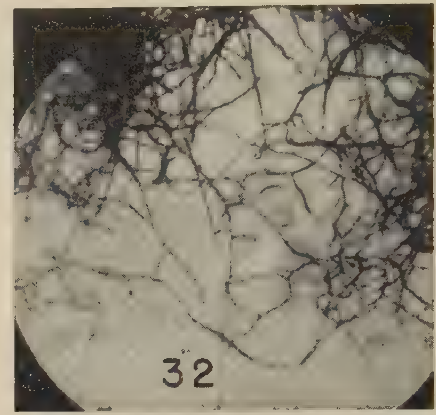


Fig. 32 *N. brasiliensis*. Three weeks incubation. 97 × objective, 10 × ocular

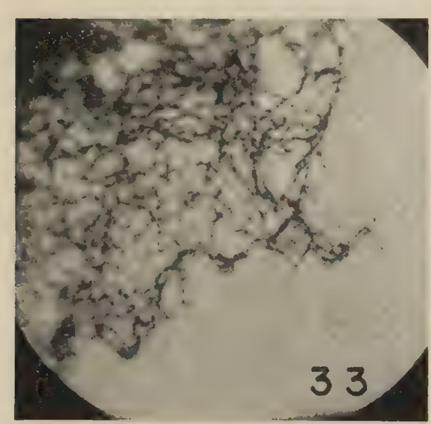


Fig. 33 *N. brasiliensis*. Six weeks incubation. 97 × objective, 10 × ocular

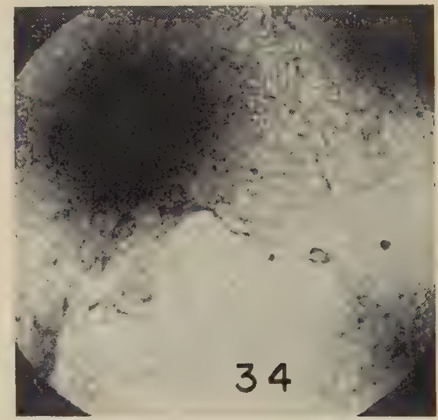


Fig. 34 *N. madurae*. One week incubation. Liquid medium. 44 × objective, 10 × ocular

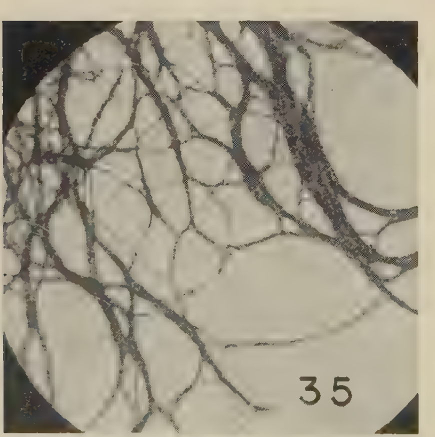


Fig. 34 *N. madurae*. One week incubation. 97 × objective, 10 × ocular

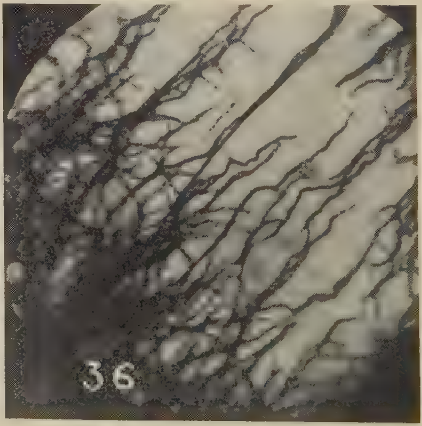


Fig. 36 *N. madurae*. Two weeks incubation. 97 × objective, 10 × ocular

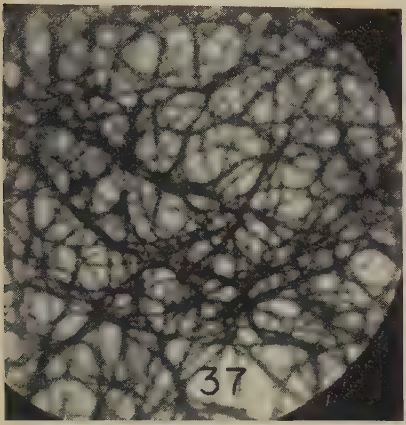


Fig. 37 *N. madurae.* Two weeks incubation. 97 × objective, 10 × ocular

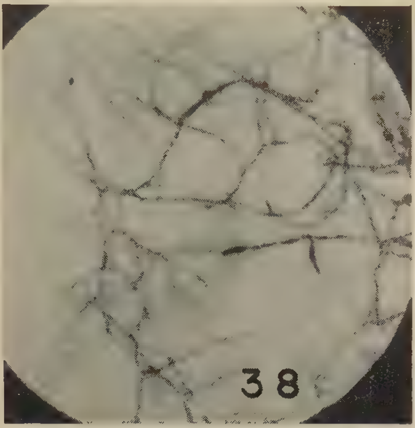


Fig. 38 *N. madurae.* Three weeks incubation. 97 × objective, 10 × ocular

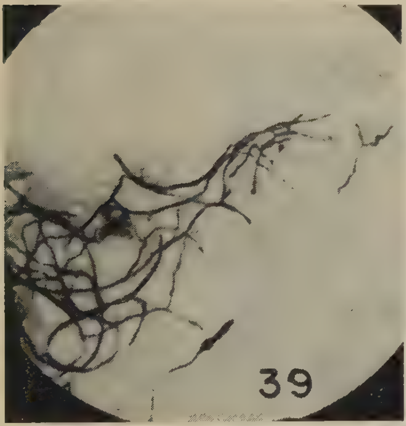


Fig. 39 *N. madurae.* Three weeks incubation. 97 × objective, 10 × ocular

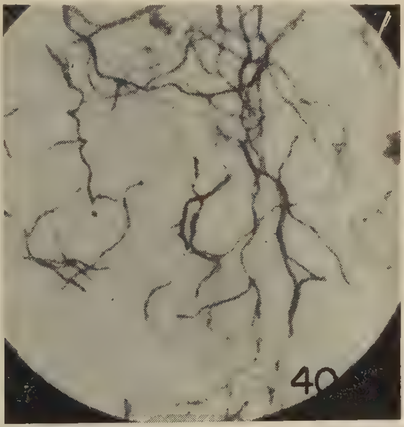


Fig. 40 *N. madurae.* Six weeks incubation. 97 × objective, 10 × ocular

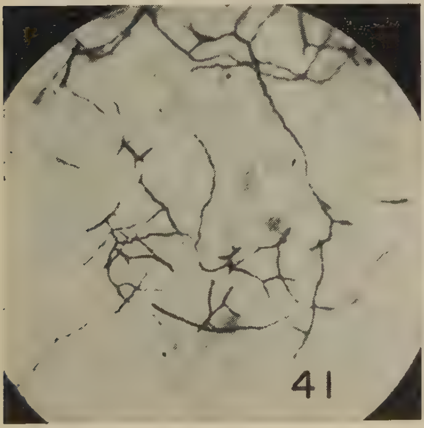


Fig. 41 *N. madurae.* Six weeks incubation. 97 × objective, 10 × ocular

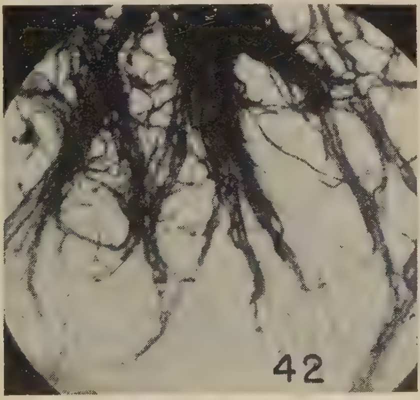


Fig. 42 *N. madurae.* One week incubation. Solid medium. 97 × objective, 10 × ocular

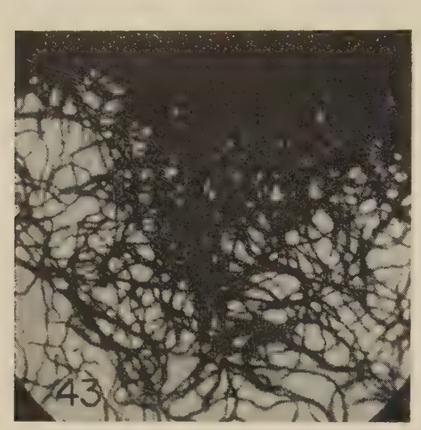


Fig. 43 *N. madurae.* Two weeks incubation.
97 × objective, 10 × ocular

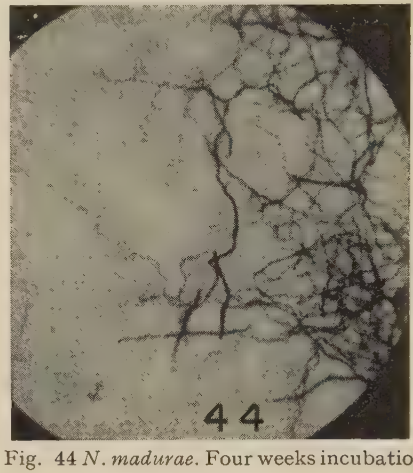


Fig. 44 *N. madurae.* Four weeks incubation.
97 × objective, 10 × ocular

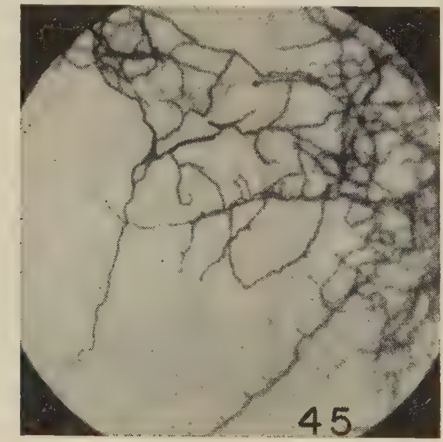


Fig. 45 *N. madurae.* Six weeks incubation.
97 × objective, 10 × ocular

characterized the preparation. Coccoid formation was observed at four weeks incubation when swelling of the tips or walls of the hyphae were seen. In some areas of this preparation the thickening of the hyphae was very noticeable. At the end of six weeks incubation, the microculture showed a preponderance of twisted hyphae which were highly branched, and fairly uniform in diameter. Terminally developing coccoid bodies were still noted but the fragmentation, observed in liquid medium, was absent (Fig. 45)).

As the microcultures were studied and compared we noted that the most definite progression of events occurred in cultures of *N. asteroides*. The growth alternated between a succession of coccoid bodies and branching hyphae with many lateral buds. We were not successful in demonstrating the fusion of hyphae or the "initial cells" described by KLIENEBERGER-NOBEL (1947). We observed that as a microcolony of *N. asteroides* aged, the center of the colony seemed to undergo degeneration and that the outer edge of the mycelial mat became thicker, had shorter branches, and showed an increase of coccoid bodies. These appeared to form between the mycelial threads. After five weeks incubation only coccoid bodies remained in the center of the colony. During the seventh week these coccoid bodies germinated in turn and the resulting hyphae were smaller and gave a weaker gram positive reaction than the primary mycelium. Since the microculture had been absorbing the nutrient material from the medium and producing metabolic end products this may be the explanation for the more delicate mycelium.

Microcultures of *N. brasiliensis* did not show this sequence. There were no free coccoid bodies in the strain that we studied. There were, among the well defined, gram positive hyphae smaller gram positive hyphae. These hyphae later lost their regular staining and developed segments of elements that were gram positive or gram negative. The culture then fragmented into bacillary forms.

The microcultures of our strain of *N. madurae* produced thick central hyphae with little branching. These central hyphae lay in parallel rows to produce strands. Some ageing hyphae showed swellings which were usually terminal. These swellings were sometimes small granules and sometimes club shaped bodies.

The three strains grew slowly as compared to ordinary bacteria. They produced an abundance of branching filaments. Although the specimens were gram positive in the aggregate some elements lost the ability to retain the crystal violet. None of the strains would have been characterized as acid-fast.

Summary

The growth patterns of *N. asteroides*, *N. brasiliensis*, and *N. madurae* had certain distinctive morphological features.

1. The branching filaments of *N. asteroides* produced an abundance of coccoid bodies and these germinated to form branching filaments.

2. The branching filaments of *N. brasiliensis* fragmented into bacillary elements that stained deeply and sometimes contained granules.

3. Young cultures of *N. madurae* produced strands of parallel hyphae and older cultures produced a small number of attached terminal coccoid or club-shaped bodies as well as swellings in the hyphae.

Bibliography

ERIKSON, D. (1948) Differentiation of vegetative and sporogenous phases of the actinomycetes, III. Variation in the *Actinomyces coelicolor* speciesgroup. J. gen. Microbiol., **2,** 252—267.

ERIKSON, D. (1949a) The morphology, cytology and taxonomy of the actinomycetes. Ann. Rev. Microbiol., **3,** 361—368.

ERIKSON, D. (1949b) Differentiation of the vegetative and sporogenous phases of the actinomycetes IV. The partially acid fast pro actinomycetes. J. gen. Microbiol., **3,** 361—368.

GONZALES-OCHOA, A. & DE LOS ANGELES-SAZDOVAL, MARIA. Caracteristicas de los actinomycetes patogenos mas communes. (1955) Rev. Inst. Salub. Enferm. Trop., **15,** 19—55.

KLIENEBERGER-NOBEL, E. (1947) The life cycle of sporing actinomycetes as revealed by a study of their structure and septation. J. gen. Microbiol., **1,** 22—37.

MCCLUNG, N. M. (1949) Morphological studies in the genus *Nocardia*. I. Developmental studies. Lloydia, **12,** 137—177.

MCCLUNG, N. M. (1950) Morphological studies in the genus *Nocardia*. II. Cytological studies. J. Bact. **59,** 589—602.

MCCLUNG, N. M. (1954) Morphological studies in the genus *Nocardia*. III. The morphology of young colonies. Ann. N. Y. Acad. Sci., **60,** 168—181.

NOVAK, M. V. & HENRICI, A. T. (1933) Pleomorphic organism showing relationships between staphylococci and actinomycetes. J. Infect. Dis., **52,** 253—267.

SPECIES OF HYPOCRELLA[1]

E. B. MAINS

(10.VII.1959)

The genus Hypocrella contains species parasitic on scale insects, Coccidae and white flies, Aleyrodidae. Many of the species were first described in the conidial condition as species of Aschersonia and it is probable that most or all of the species of Aschersonia are conidial stages of species of Hypocrella. PETCH (1921) has published a comprehensive treatment of the two genera. In a study of the species of tropical America additional information has been obtained concerning some of the species which is presented here.

Types and other specimens were made available for this study from the Farlow Herbarium (FH) by J. MACKENZIE LAMB; from the Herbarium of the New York Botanical Garden (NY) by CLARK T. ROGERSON; from the Mycological Collections, U. S. Bureau Plant Industry (BPI) by JOHN A. STEVENSON; from the Herbarium of the Florida Agr. Exp. Sta. (FLAS) by ERDMAN WEST; from the Herbarium of the University of California (UC) by HERBERT L. MASON; from the Naturhistoriska Riksmuseet of Sweden (S) by T. E. HASSELROT; from the Instituto de Botanica Spegazzini (LPS) by JUAN C. LINDQUIST; and from the Herbarium of the Instituto Agronomico do Estado de São Paulo (IAC) by A. P. VIÉGAS.

Hypocrella caulium (BERK. & CURTIS) PAT. Bull. Soc. Mycol. Fr. 30 : 346. 1914. *Corticium caulium* BERK. & CURTIS. J. Acad. Nat. Sci. Philad. 2 : 279. 1854. *Hypocrella camerunensis* var. *brasiliana* P. HENN. Hedwigia 43 : 85. 1904.

This species is fairly common in the American tropics. It usually is collected in a sterile condition and PATOUILLARD (1914) and PETCH (1921) concluded that it was first described by BERKELEY & CURTIS from sterile stromata from Surinam, as *Corticium caulium.* A portion of the type collection in the Curtis Collection of the Farlow Herbarium does not contain a fungus. R. W. G. DENNIS informs me that the part of the type in the Kew Herbarium is sterile and appears to be the fungus treated by PETCH as *Hypocrella caulium.* PATOUILLARD transferred *C. caulium* to Hypocrella on the supposition that it was conspecific with *H. camerunensis* P. HENN. from Africa. HENNINGS also described a variety, H. *camerunensis* var.

---

[1] Paper from the Herbarium and the Department of Botany, University of Michigan, Ann Arbor, Michigan.

brasiliana from Brazil. This was based on a collection by E ULE No. 2613 from Rio de Janerio, Brazil. Perithecia and asci are described. A portion of this collection is in the Farlow Herbarium. It consists of three scutate stromata which are 11—16 mm wide and dull brownish red. They are sterile and are similar to sterile stromata of *H. caulium*. PETCH (1921) states that the portion of the type in the Kew Herbarium is also sterile. Apparently only a few of the stromata of the type collection had perithecia and it is doubtful if they now exist.

A collection by CARLOS E. CHARDON, No. 4065, from Venezuela, (BPI) has perithecia which agree with the description given by HENNINGS for *H. camerunensis* var. *brasiliana*. The stromata (fig. 1) are scutate to pulvinate, 8—20 mm wide. The perithecia are produced in yellowish to light gray cushions on the flat orange stromata. The perithecia are ovoid, 225—350 × 132—150 μ and the asci somewhat fusoid, 175—250 × 7—10 μ, having caps 2—4 μ thick. The ascospores are filiform nearly as long as the asci, 1.5—2 μ wide, multiseptate with cells 6—12 μ long. Part spores were not seen. This appears to be the same as that described by HENNINGS as *H. camerunensis* var. *brasiliana*.

HENNINGS (1897) described *H. camerunensis* from a collection by STAUDT No. 214 from Lolodorf, Cameroon, West Africa. The type has not been seen and probably does not exist. There is a specimen in the Farlow Herbarium No. 4101 collected by SCHWAB from Cameroon West Africa and identified as *H. camerunensis*. This has coalesced stromata (fig. 2) which are irregularly hemispherical 10—13 mm wide and 5—10 mm thick. The light yellow perithecia are produced in orange hemispherical to flat cushions and are ovoid, 250—330 × 130—150 μ. The asci are cylindric 150—160 × 4 μ and immature. HENNINGS describes the asci as cylindric, 180—250 × 3.5—4.5 μ and the ascospores as breaking into segments 12—15 × 0.3 μ The variety *brasiliana* differs in the yellowish to light gray, flat perithecial cushions of the stroma, the wider asci and ascospores which do not break into segments. It therefore is best treated as a species. The variety *brasiliana* is the first name given to the perfect stage and therefore *Hypocrella brasiliana* (HENN.) comb. nov. is proposed for the species.

Hypocrella turbinata PETCH Ann. Roy. Bot. Gard. Peradeniya 5 : 535. 1914.

THAXTER (1914) described and illustrated the perithecial stage of *Aschersonia turbinata* from a collection which he made in Grenada without including the species in a genus of the Ascomycetes. PETCH placed it in Hypocrella referring to the description by THAXTER. There is a collection No. 4272, under *Aschersonia turbinata* in the Farlow Herbarium which was made by R. THAXTER at Grand Etang Grenada, 1912—1913, accompanied by notes and sketches which apparently were the bases for his publication. It therefore is the lectotype of the species. Although the species is common in

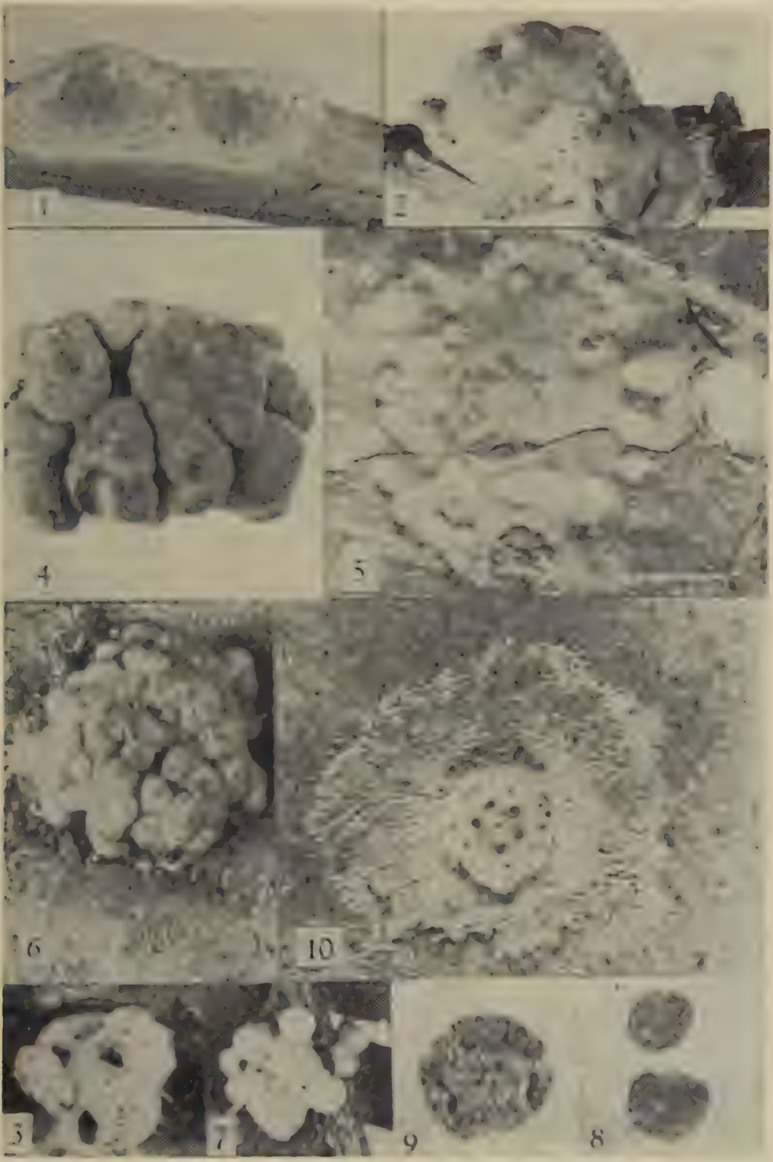


Fig. 1, *Hypocrella brasiliana*, Chardon 4065, stromata, side views, 10 ×
Fig. 2, *H. camerunensis*, FH4101, stromata, side views, 10 ×.
Fig. 3, *H. turbinata*, FH4272, stroma top, view, 10 ×
Fig. 4, *H. Schizostachyi*, type, troma, side view, 5 ×
Fig. 5, *H. libera*, FH3951, stroma, top view, 10 ×
Fig. 6, *H. sloaneae*, type, stroma, top view, 10 ×
Fig. 7, *H. nectrioides*, FH3996, stroma, top view, 10 ×
Fig. 8, *H. castanea*, FH4247, two stromata, top view, 10
Fig. 9, *H. viridans*, FH4263, stroma, top view, 10 ×
Fig. 10, *H. citrina*, Herb. Speg. 904, top view, 10 ×.

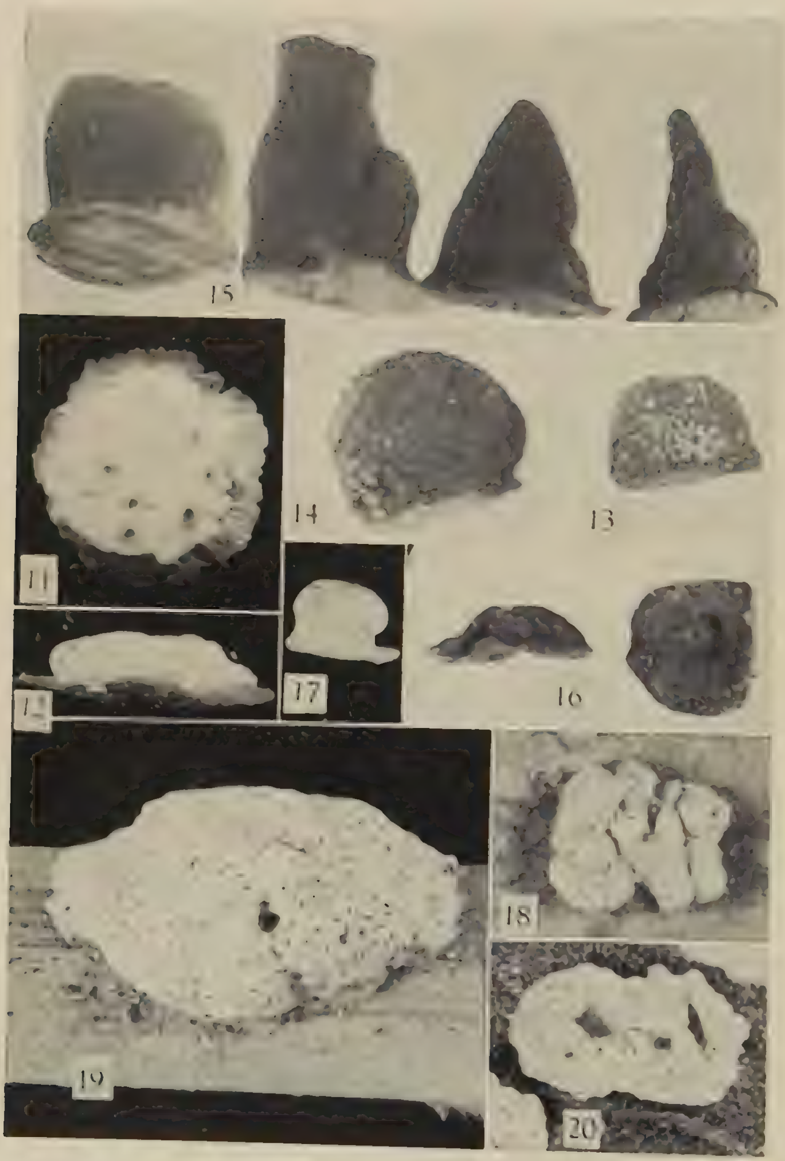


Fig. 11 and 12, *H. aurantiaca*, type, stromata, 11, top view, 12, side view, 10 ×.
Fig. 13, *H. guaranitica*, type, stroma, side view, 10 ×
Fig. 14, *H. orbicularis*, Noack FH, stroma, side view, 10 ×.
Fig. 15, *H. cornuta*, type, four stroma showing variations, side views, 5 ×.
Fig. 16, *H. melaena*, type, stromata, side and top views, 5 ×.
Fig. 17, *H. disjuncta*, type, stroma, side view, 10 ×.
Fig. 18, *H. colliculosa*, type, stroma, top view, 10 ×.
Fig. 19, *H. andropogonis*, FH6257, stroma, side view, 10 ×
Fig. 20, *H. amazonica*, type, stroma, top view, 10 ×.

the American tropics in the Aschersonia stage only, two other collections made by C. L. LINDER, Nos. 7949 and 861a, in British Guiana (NY) with the perithecial stage have been seen.

The species is distinguished by having the pycnidia produced in one or more flat or concave disks on a more or less bulbose base (fig. 3 also THAXTER figs. 1—7). The pycnidia are irregular, labrinthiform and the pycnidiospores fusoid, 10—12 × 3—4 μ. Paraphyses are not produced. The perithecia develop in one or more swellings or cushions usually associated with pycnidial disks. They are flask-shaped, 400—600 × 150—250 μ with well defined walls up to 30 μ thick. The asci are cylindric or somewhat fusoid, 200—250 × 8—12 μ with 3—4 μ thick caps. The part-spores are cylindric, 8—14 × 2—3 μ, with rounded ends.

Hypocrella schizostachyi P. HENN. Philad. J. Sci. 3 : 45. 1908.

HENNINGS described this species from stromata collected by FOXWORTHY on Schizostachyum from Rizal, Luzon, Philippines, Phil. Bur. Sci. No. 46. Parts of the type collection are in the Herbarium of the New York Botanical Garden and in Mycological Collections, U. S. Bureau Plant Industry. The stromata (fig. 4) are irregularly globoid, 6—10 mm wide, irregularly tuberculate to cerebriform, grayish brown, purple in KOH. The perithecia are scattered, completely embedded and flask-shaped, 400—500 × 175 —230 μ with walls up to 35 μ thick. The asci are cylindric, 170—205 × 6 μ with 3 μ thick caps. The ascospores break into cylindric part-spores, 4—8 × 1.5—2 μ. HENNINGS describes the stromata as 1.5—2 cm wide.

This species is very similar to *H. gartneriana* MÖLLER from Brazil which differs mostly in the light yellow stromata which do not change to purple when treated with KOH.

Hypocrella libera SYD. Ann. mycol. 14 : 85. 1916.

This species was described by SYDOW as on coccids from a collection by E. ULE, No. 3413, from Cobija, Rio Acre, Bolivia. The type has not been seen and probably does not exist. It is not among the specimens of the Sydow Herbarium at Stockholm. PETCH (1925) gives a description which apparently was made of the type. He also concludes that the pycnidial stage is *Aschersonia aleyrodis*. This conclusion was mostly based on two collections on scale insects by J. R. JOHNSTON from Changuinola, Panama received from Professor THAXTER. One (No. 3951) occurs under *Aschersonia* and the other (No. 4099) as *Hypocrella aleyrodis*. in the Farlow Herbarium. The stromata (fig. 5) are thin, flat to slightly scutate, 2—5 mm wide and tuberculate. The perithecia occur singly in the tubercules mostly on the margins of the stromata and are immature. The pycnidia are poorly developed in the center of the stromata and the pycnidiospores are fusoid, 10—14 × 1.5 μ. Paraphyses were not seen. The hosts are probably Aleyrodidae.

Hypocrella libera is very similar to *H. raciborskii* of the eastern hemisphere as treated by PETCH (1921). It has similar tuberculate stromata. The pycnidial stage, *Aschersonia placenta* is also very similar to *A. aleyrodis*. It is doubtful if the two should be recognized as distinct species. However it is questionable whether *H. raciborskii* described by ZIMMERMANN is the same as the species reported by PETCH. ZIMMERMANN (1901) does not illustrate a tuberculate condition and he states that the perithecia are entirely embedded.

Hypocrella sloaneae PAT. in DUSS, Enumeration Champignons Guadeloupe et Martinique p. 80. 1903.

The type of *Hypocrella sloaneae* was collected by DUSS on Sloanea in Guadeloupe in 1903. It is in the PATROUILLARD Collection of the Farlow Herbarium. The stromata (fig. 6) are pulvinate, 1—3 mm wide, light yellowish brown, tuberculate with a slight hypothallus. The tubercules are crowded over the entire stroma. The perithecia occur singly in the tubercules and are ovoid, 350—450 × 200 μ. The asci are somewhat fusoid, 300—350 × 14—16 μ with 6 μ thick caps. The part-spores are fusoid, 8—12 × 2 μ, acute at the ends. Pycnidia do not occur.

PETCH (1921) has described a pycnidial stage for *H. sloaneae*. His description and illustrations were taken from the type of *H. amazonica* which he treates as a synonym. *H. amazonica* is somewhat verrucose, not tuberculate, and the asci cylindric and smaller (see following). *H. nectrioides* and *H. sloaneae* are very similar. According to PETCH the stromata of *H. nectrioides* are rosy orange when fresh. There is no information concerning the color of stromata of *H. sloaneae* in the fresh condition. In the dried condition those of *H. nectrioides* are white and those of *H. sloaneae* are light yellowish brown. According to the description given by PETCH (1921) *H. amomi* of the eastern hemisphere is also very similar to *H. sloaneae*.

Hypocrella nectrioides THAXTER ex PETCH, Ann. Roy. Bot. Gard. Peradeniya 7 : 225. 1921.

PETCH described this species from collections by R. THAXTER on scale insect on *Pentaclethra* sp. St. Ann's Valley, Port of Spain, Trinidad. There are two collections in the Farlow Herbarium, Nos. 3996 and 4296, collected by THAXTER on Cocci on *Pentaclethra*, Maraval Valley, Port of Spain, Trinidad, and determined by PETCH as *Hypocrella nectrioides*. They have pulvinate, white, tuberculate stromata (fig. 7) ,1—2 mm wide. The tubercules are crowded over the entire stroma. The perithecia occur singly in the tubercules and are ovoid to flask-shaped, 450—500 × 300—350 μ with walls up to 35—40 μ thick. The asci are somewhat fusoid, 300—350 × 12—16 μ with 4—6 μ caps. The part-spores are somewhat fusoid or asymetric, 9—16 × 2—3 μ, with rounded ends. Pycnidia do not occur.

PETCH (1925) later concluded that *H. nectrioides* was the same as *H. libera.* The stromata of the later are flat and thinner with the tubercles mostly developed on the margin. *H. nectrioides* appears to be closer to *H. sloaneae.*

Hypocrella castanea PETCH, Trans. Brit. Mycol. Soc. 16 : 224. 1932.

This species was described by PETCH from specimens collected by R. THAXTER on an aleyrodid on *Adiantum* sp. from St. Ann's, Port of Spain, Trinidad. PETCH previously (1921) had determined the collections as *H. palmicola* and his figs. 31 and 32, Pl. 3 of that species are of *H. castanea.* There are three specimens in the Farlow Herbarium Nos. 4089, 4245, and 4247 collected by THAXTER in Trinidad and determined by PETCH as *H. palmicola.* Two, 4245 and 4247 are given as from St. Ann's Valley, No. 4247 is in the best condition and should probably be considered the lectotype of the species.

The stromata (fig. 8) are discoid or flattened pulvinate, 1 mm wide, dark brown. The perithecia are flask-shaped, 200—250 × 100—130 μ, with walls 20 μ thick. The asci are slender, cylindric, 70—100 × 3—4 μ and the walls are not thickened into a cap. The ascospores are broken into cylindric one-celled fragments, 4—7 × 1.5 μ, in the asci. The narrow asci and few part-spores indicate that the asci probably are two or four-spored. Pycnidia were not seen. PETCH describes them as central and single in the stromata as shown by his fig. 32, pl. 3 (PETCH (1921)). The pycnidiospores are given as fusoid, 4—6 × 1 μ, ends not produced and the paraphyses as up to 130 μ long.

Some of the stromata of No. 4245 in the Farlow Herbarium have sterile horn-like projections.

Hypocrella viridans PETCH, Ann. Roy. Bot. Gard. Peradeniya 7 : 236. 1921.

This species is usually collected in the pycnidial stage and occurs in herbaria as *Aschersonia viridans* (BERK. & CURTIS) PAT. The perithecial stage was described by PETCH from collections by THAXTER obtained in Trinidad. Collections Nos. 4261, 4262, 4263, 4264, 4267, 4025 and 4086 in the Farlow Herbarium under *Hypocrella viridans* and *Aschersonia viridans* having perithecia were collected by THAXTER in Trinidad, 4261, 4263 and 4068 being determined by PETCH. The stromata (fig. 9) are discoid, circular in outline or sometimes slightly crenulate, 1—2.5 mm wide, green,punctate from the scattered ostioles of the perithecia with a slight to broad hypothallus. The perithecia are scattered, flask-shaped, 300—350 × 150—200 μ. The necks are surrounded by an obconic tissue up to 200 μ wide of longitudinal, thin-walled hyphae. The asci are somewhat fusoid, 120—175 × 6—7 μ with 2 μ thick caps. The ascospores are filiform attenuated below, 50—100 × 2—2.5 μ, multisepatate, the cells 16—20 μ long. Part spores were not seen. Pycnidia usually accompany the perithecia often in a peripheral circle. They are globoid to flattened globoid, 200—400 μ wide, usually with moderately wide orifices. The pycnidiospores are fusoid, 10—16 × 2 μ with acute ends. The paraphyses are 60—150 μ long.

H. citrina is a very similar species differing mostly in having yellow stromata. *H. discoidea* of the eastern hemisphere with red stromata is also closely related.

Hypocrella citrina SPEG. Bol. Acad. Cienc. Cordoba 11 : 537. 1889.

This species was described from a collection by J. PUIGGARI, No. 2538, from Apiahy, S. Paulo, Brazil, 1888. The type in the Herbarium Spegazzini No. 904 has been studied. The stromata (fig. 10) are discoid or flattened pulvinate, 0.5—2.0 mm wide, light yellow, punctate from the brownish ostioles and with broad fibrose hypothalli. Mature perithecia are few, globoid with short ostioles, 250—300 μ wide. The asci are somewhat fusoid or clavate, 120—150 × 6—8 μ, with multiseptate, filiform ascospores, 100—110 × 1.5 μ not breaking into segments. Most of the stromata bear only pycnidia but a few also have perithecia. The pycnidia are oblate spheroid constricted above into narrow ostioles, 400—500 × 400—450 μ. The pycnidiospores are fusoid, 12—16 × 2 μ and the paraphyses filiform up to 200 μ long.

THEISSEN (1911) concluded that this species was the same as *H. ochracea* MASSEE which PETCH (1921) considers to be *H. phyllogena*. However, it is distinct from the latter in both the perithecial and pycnidial stages. The Aschersonia stage is *A. flavocitrina*.

Hypocrella aurantiaca (PETCH) comb. nov. *Stereocrea aurantiaca* PETCH, Trans. Brit. mycol. Soc. 23 : 136. 1939.

This species was described by PETCH as *Stereocrea aurantiaca* from a collection on the aleyrodid *Paraleyrodis perseae* from Gainesville, Florida sent to him by ERDMAN WEST. The type collected by WEST and VOORHEES No. 11876 has been studied from specimens in the Herbarium of the Florida Agr. Exp. Sta. (21166) and in Mycological Collections U. S. Bureau Plant Industry. The stromata (figs. 11 & 12) are discoid, 1—2 mm wide, orange-yellow, smooth or verrucose, with narrow somewhat fimbriate hypothalli. The asci are fusoid, 150—200 × 12—16 μ with 4—6 μ caps. The ascospores are narrowly fusoid or clavate, 110—150 × 4—5 μ, multiseptate not breaking into fragments. Pycnidia occur with the perithecia. The pycnidiospores are fusoid 20—34 × 4—5 μ, with acute ends. The walls are slightly thickened at the ends, 1—2 μ. The paraphyses are filiform, up to 150 μ long.

PETCH noted the similarity to species of Hypocrella but decided that the fusoid asci and the fusoid, non-fragmentating ascospores excluded it. These characters are found in various degrees in species of Hypocrella and it is therefore included in that genus.

Hypocrella palmae (BERK. & CURTIS) SACC. Syll. fung. 2 : 580. 1883. *Hypocrea palmae* BERK & CURTIS, J. Acad Nat. Sci. Philad. 2 : 285. 1854.

BERKELEY & CURTIS described this species from specimens on leaves of palm from Surinam from the Schweinitz Herbarium. There is a specimen in the Curtis Collection of the Farlow Herbarium which is part of the type. It consists of one stroma which is hemispherical, 2 mm wide and brownish black. The perithecia are immature. BERKELEY & CURTIS state that the perithecia are "scarcely mature". PETCH (1921) states that the type specimen in the Kew Herbarium has two stromata which are two-thirds globose 2 and 4 mm wide, black and with perithecia peripheral and part-spores 8—9 × 2 μ. This species as treated by PETCH is very variable especially in the shape of the stromata and he lists ten synonyms. Of these *H. spegazzini* SACC., *N. guaranitica*, SPEG., *H. globosa* SYD., *H. orbicularis* SYD., and *H. sydowii* SACC. & TROTTER are discussed here. PETCH (1921) describes the stromata of *H. palmae* as hemispherical or two-thirds globose, up to 6 mm diameter, red-brown becoming black and the perithecia as peripheral, flask-shaped 300—400 × 200 μ with asci 160 × 8 μ and part-spores cylindric, 5—10 × 1.5—2 μ, with rounded ends. His description of pycnidia is based on a collection by THAXTER from Trinidad which does not appear to be *H. palmae*.

Hypocrella reineckiana P. HENN. one of the most common species of the eastern hemisphere is probably conspecific with *H. palmae*. A comparison of the descriptions given by PETCH shows a very close similarity.

Hypocrella guaranitica SPEG. Fungi Guaranitici Pugillus 1. No. 256. 1883.

This species was described from a collection by B. BALANSA, No. 3546 from Villa-Rica, Paraguay. The type is in the Herbarium of the Instituto Spegazzini, No. 901. Part of the type collection is also in the Herbarium of the New York Botanical Garden. The stromata (fig. 13) are subspherical or hemispherical, 1—2 mm wide, dark chocolate brown or black. The perithecia are flash-shaped, 400—450 × 200—250 μ with walls 30 μ thick and the asci cylindric, 175—225 × 6—8 μ, with walls not or only slightly thickened at the apices. The part-spores are cylindric, 4—8 × 1.5 μ, rounded at the ends. Pycnidia were not found. As PETCH (1921) has concluded this is conspecific with *H. palmae*.

Hypocrella spegazzini SACC. Syll. fung. 2 : 579. 1888.

SACCARDO based this species on a collection by J. PUGGARI No. 1483 from Apiahy, S. Paulo, Brazil, published by SPEGAZZINI (1881) as *H. phyllogena* (Fungi Argentini 209). The type, No. 903 in the herbarium of the Instituto Spegazzini, has flattened pulvinate, brownish black stromata, 1—5 mm wide. The perithecia are flask-shaped 400—500 × 150 —300 μ, with walls up to 35 μ thick. The asci are agglutinated and uncertain. Notes and sketches on the packet by SPEGAZZINI give the asci as cylindric to clavate, 160—200 × 10—15 μ and the part-spores as cylindric 3—8 × .75—1 μ with rounded ends. PETCH (1921) has concluded that this is *H. palmae.* The stromata are flatter than usual for *H. palmae* and the asci as given by SPEGAZZINI are wider.

Hypocrella orbicularis SYD. in THEISSEN, Ann. mycol. 9:67. 1911.

This species was described from a collection by F. NOACK, No. 675, from São Francisco dos Campos, São Paulo, Brazil and originally named *H. globosa* by the SYDOWS (1907). RACIBORSKI had previously in 1906 named a species from Java, *Hypocrella globosa. H. globosa* SYD. was also independently renamed *H. sydowii* by SACCARDO & TROTTER (1913). There is a collection of *H. globosa* SYD. in the Farlow Herbarium from the herbarium of THEISSEN collected by NOACK at São Paulo, Brazil which probably is part of the type. It consists of three black globoid stromata 3 mm wide (fig. 14). The perithecia are flask-shaped, 350—400 × 200 μ with walls 30 μ thick. The asci are agglutinated and uncertain. *H. orbicularis* appears to be *H. palmae* as concluded by PETCH (1921). *H. globosa* RAC. is probably *H. reineckiana* according to PETCH (1921) and it is doubtful if the latter is distinct from *H. palmae.*

Hypocrella cornuta H. P. KRUG in VIEGAS Bragantia 4 : 93. 1944. nom. nudum.

VIEGAS listed this species for Brasil and stated that it had not been described and he informs me that it has not since been described. The reare several specimens Nos. 2900, 3692 and 5129 so named in the herbarium of the Instituto Agronomico do Estado, São Paulo, Campinas, Brasil. Most of the stromata (fig. 15) are more or less conoid, some laterally flattened and a few hemispherical, 2—6 mm wide and 3—7 thick. They are dark brown to black. The perithecia are flask-shaped, 300—400 × 150—200 μ with walls 20—30 μ thick. The asci are cylindric, 150—200 × 7—8 μ with the walls slightly thickened at the apices. The ascospores break into somewhat fusoid part-spores, 8—10 × 1.5 μ, rounded at the ends. Pycnidia were not found.

This appears to be a variant of *H. palmae.* It differs principally in the conoid shape. However, some hemispherical stromata occur (fig. 15).

Hypocrella melaena SYD. Philad. J. Sci. 8 Sec. C : 494. 1913.

This species was described from a collection by RAMOS, Phil. Bur. Sci. No. 20614, from San Antonio, Prov. Laguna, Philippines, February 1913. Portions of the type collection in the Herbarium of the New York Botanical Garden and in Mycological Collections, Bureau

Plant Industry, Washington, D. C. (General Collection and Lloyd Herbarium 5919) have been studied. The stromata (fig. 16) are flat, scutate, 3—12 mm wide and black. The perithecia are flask-shaped, 450—500 × 250—300 μ. The mature asci are fusoid or narrowly amphoric, 155—175 × 10—14 μ, narrowing to 5—6 μ above, the walls not or slightly thickened 1—1.5 μ at the apices. The part-spores are cylindric, 4—6 × 1.5 μ rounded at the ends. Pycnidia were not found. Petch (1921) considers this species as conspecific with *H. reineckiana* which has subspherical stromata and cylindric asci. The flat scutate stromata and fusoid to narrowly amphoric asci justify the recognition of *H. melaena* as a separate species. The specimen of Thaxter from Trinidad upon which Petch (1921) based his description of pycnidia of *H. palmae* appears to be this species.

Hypocrella phyllogena (Mont.) Speg. Fungi Argentini No. 209. 1881. *Hypocrea phyllogena* Mont. Ann. Sci. nat. ser. II, 13 : 340. 1840.

Hypocrea phyllogena was described and illustrated by Montagne from a collection No. 580 by Leprieur from Cayenne, French Guiana. Spegazzini, in transferring the species to *Hypocrella* misapplied the name to *H. palmae*. Montagne described the stromata as orange, smooth, hemispherical or pulvinate, constricted at the base (Montagne figs. 4 o & p). The ascus is illustrated (Montagne fig. 4s) as somewhat fusoid and the part-spores (fig. 4t) as allantoid with a slight swelling in the middle. According to Petch (1921) there is only one stroma in the type in the Montagne Herbarium. The shape of the stromata given as studshaped by Petch and the allantoid part-spores with middle swellings are unusual. In a study of collections having these characters it has been found that the shape of the ascus differs somewhat from that given by Montagne. It is more fusoid to narrowly amphoric being shaped like a narrow amphora gradually narrowing below from a width of 10—16 μ and somewhat abruptly narrowing above to a more or less cylindric neck.

The stromata of the species as treated by Petch are more variable than shown by Montagne. Although he distinguished the species principally by having studshape stromata he states that the stromata are also pulvinate, conical or conico-convex. Montagne illustrated the stromata as smooth. Petch has concluded that *Moelleria sulphurea* Bres. with verrucose stromata (Petch 1921 pl. 2 fig. 17) is conspecific.

Picnidia have been found associated with perithecia. In the studshaped stromata the perithecia open on the surface of the head and the pycnidia are mostly found in the stipe. In the pulvinate stromata the perithecia are mostly in the center and the pycnidia in the margin. The pycnidiospores are distinctive. They are fusoid, 8—14 × 2 μ with the walls at the ends attenuately thickened, 2—4 μ. Studshaped stromata frequently occur having only pycnidia. In this condition the species was described as *Aschersonia basicystis* Berk. & Curtis.

Hypocrella verruculosa Möller. Phycomyceten und Ascomyceten p. 299. 1901.

This species was apparently described from one stroma from Blumenau, Brazil. According to Petch (1921) it was preserved in alcohol at Berlin. It probably is not now in existance. As described and illustrated by Möller the stroma was subgloboid, 7 mm wide, yellowish brown and very verrucose. The ascus is illustrated as clavate without a cap and described as 600 μ long and 4-spored. The part spores are illustrated as narrowly ellipsoid and described as 12—15 × 3—5 μ. Pycnidia are not described.

Theissen (1911) has reported a collection made by Rick in Brazil as this species. This has been distributed as No. 294 of Rick's Fungi Austro-americani. It is a mixture of two species. The flattened pulvinate, grayish-brown smooth stromata appear to be an undescribed species, The stromata of the other species are hemispherical to studform, 2—3 mm wide, 1.5—2 mm thick, light brownish yellow, more or less verrucose. The perithecia are flask-shaped, 450—500 × 250—300 μ, with walls up to 50 μ thick. The asci are fusoid to narrowly amphoric, 200—250 × 12—16 μ, with walls not thickened into a cap. The part-spores are cylindric somewhat fusoid or allantoid, 10—14 × 2—4 μ, with rounded ends. It differs from *H. verruculosa* as described by Möller in the shape of the stromata, size and shape of the part-spores. As Petch (1921) has concluded it is the verrucose form of *H. phyllogena*. *H. verruculosa* has also been reported from Brazil by Viegas (1944) for a collection by H. P. Krug (JAC 2894). It is only in pycnidial condition and the identity is doubtful.

Hypocrella disjuncta Seaver, Mycologia 12 : 97. 1920.

This species was based on a collection by Johnston and Stevenson No. 1640 from Naguabo, Puerto Rico. A brief description is given by Seaver who mentions only asci and ascospores. The following description is from the type in the Herbarium of the New York Botanical Garden and a part of the type in Mycological Collections U. S. Bureau of Plant Industry.

The stromata (fig. 17) are hemispherical, studform or pulvinate, smooth or slightly verrucose, 1—2 mm wide, yellowish, punctate on the head or in the center from the ostioles of the perithecia, with orifices of the pycnidia round or irregular on the shanks or margins of the stromata. The perithecia are flask-shaped, 350—400 × 200—250 μ, with walls up to 50 μ thick and the asci fusoid or narrowly amphoric, 150—230 × 10—12 μ, with 2 μ thick caps. The ascospores break into 1-celled allantoid to somewhat fusoid part-spores, 5—10 × 1.5—2 μ, frequently with a median swelling on the concave side and with ends rounded. The pycnidia are irregular, up to 400 μ wide and the pycnidiospores fusoid, 5—8 × 1.5 μ. Paraphyses are lacking. The host is given as white fly. It however appears to be a scale insect which is substantiated by the lack of paraphyses.

PETCH (1925) concluded from the description given by SEAVER that *H. disjuncta* was conspecific with *H. phyllogena*. They are very similar but differ in the pycnidiospores which were not described by SEAVER. The pycnidiospores of *H. phyllogena* are larger, 10—14 × 2 μ with the walls at the ends attenuately thickened 2—5μ.

Hypocrella colliculosa SPEG. Fungi Puiggariani No. 301. 1889.

This species was described from a collection by J. PUIGGARI No. 2339 from Apiahy, S. Paulo, Brazil. The type from the Herbarium Spegazzini No. 902 has been studied. The stromata (fig. 18) are pulvinate, 0.5—2.5 mm wide, irregularly verrucose to colliculose and glabrous. SPEGAZZINI describes them as "pallide subaurantia". They are now light brown. The perithecia are flask-shaped, 400—500 × 200—300 μ with walls 25—30 μ thick and the asci cylindric, 200 × 8—10 μ, with 2 μ thick caps. The part-spores are cylindric, 6—10 × 2—2.5 μ, with rounded ends. Pycnidia were not found. The host is uncertain.

Hypocrella fluminensis KRUG, Jornal Agronomia 3 : 75. 1940.

This species was described from a collection by FELISBERTO DE CAMARGO from Theresopolis, Prov. Rio de Janeiro, Brazil. The type is in the Herbarium of the Instituto Agronomico do Estado de São Paulo, Campinas, Brazil, No. 2871. Only a few stromata occur. They are pulvinate, 1.5—2 mm wide and are brown to dark gray. There is a large central orifice. The pycnidia are irregularly lobed. They are given as 654—1140 × 715—836 μ by KRUG. The pycnidiospores are fusoid, 9—12 × 2.5—4 μ, with acute ends. No paraphyses were found as was also noted by KRUG. It appears to be *Aschersonia cubensis*.

In the one stroma examined perithecia were not found. KRUG states that they occur in the basal part of the stoma. He describes them as flask-shaped, 350—470 × 167—243 μ and the asci as fusiform, 182—243 × 10—16 μ with the wall not or only slightly thickened at the apices. The part-spores are described as ovate-oblong, 7—11.5 × 2—3 μ. The hosts are given as Aleyrodidae. This is questionable. The lack of paraphyses in the pycnidia indicate that the hosts probably are Coccidae. Both in the pycnidial and perithecial stages the fungus agrees very well with *H. epiphylla*.

Hypocrella andropogonis PETCH, Ann. Roy. Bot. Gard. Peradeniya 7 : 247. 1921.

PETCH described the perithecial stage of *Aschersonia andropogonis* P. HENN. from a collection made by THAXTER in Trinidad. A collection No. 6257 in the Farlow Herbarium determined by PETCH as *H. andropogonis* appears to be part of the type specimen to which PETCH refers. The stromata (fig. 19) are pulvinate, 2—6 mm wide, white to yellowish, even or somewhat verrucose, punctate from the brownish ostioles of the perithecia which are flask-shaped, 400—450 × 180—230 μ with walls 20—25 μ thick. The asci are cylindric, 200—250 × 6—8 μ with 2.5—3 μ thick caps. The part-spores are cylindric, 10—14 (18) × 2 μ with rounded ends. Pycnidia occur associated with the perithecia. They are broadly ovoid with large conspicuous orifices. The pycnidiospores are fusoid, 8—10 × 1.5 μ with acute ends and the paraphyses are filiform up to 250 μ long

This species is very similar to *H. cretacea* v. HÖHN. of the eastern hemisphere (see following).

Hypocrella cretacea v. HÖHN. S. B. Kl. akad. Wiss. Wien 118 : 311. 1909.

This species was described from a collection made by VON HÖHNEL on *Amomum* sp. in Tjibodas Java. It appears to have been distributed by REHM in his Ascomycetes No. 1870. The stromata are pulvinate, 1.5—5 mm wide and 1—2 mm thick, punctate from the brown ostioles of the perithecia which are flask-shaped, 400—500 × 200—250 μ with walls 30 μ thick. The asci are cylindric, 150—200 × 6—8 μ with 2 μ thick caps. The ascospores are filiform with cells 6—8 × 2 μ. Pycnidia were not found.

H. cretacea therefore is very similar to *H. andropogonis*. PETCH (1921) however has concluded that *H. cretacea* is the same as *H. mollii* (see following) for which he describes pycnidia which are flask-shaped, 300 × 160 μ.

Hypocrella mollii KOORD. Verh. akad. Wet. Amst., Tweede Sect. 13 : 179. 1907.

This species was described from specimens on *Premna tomentosa* from Kaliwiro, Kedu, Java collected by KOORDERS. There is a specimen in the Farlow Herbarium labeled "authentic" which appears to be part of the type collection. The stromata are slightly pulvinate, 1—2 mm wide, soft, white to light cream, slightly pubescent. The perithecia are ovoid, 350—450 × 200—220 μ and the asci cylindric or slightly fusoid, 175 × 6—9 μ with 3—4 μ caps. The ascospores are filiform 1—1.5 μ thick. Part-spores were not seen. KOORDERS describes them as 15—17 μ long. Pycnidia were not found.

There are two specimens of *H. mollii* in the Farlow Herbarium collected and determined by PETCH. Number 4097 which only has perithecia is very similar to the type of *H. cretacea*. Number 4096 only has pycnidia (*Aschersonia confluens*) which are flask-shaped 125—150 × 60—75 μ with fusoid spores, 8—12 × 1.5—2 μ.

In the discussion of *H. mollii* PETCH (1921) does not state that he found perithecia and pycnidia together. It is doubtful whether *H. mollii* and *H. cretacea* are the same and whether the pycnidia described belong to either.

Hypocrella amazonica P. HENN. Hedwigia 43 : 246. 1904.

This species was described by HENNINGS from a collection of ULE, No. 3198, from Iquitos, Peru. A part of the type is in the Farlow Herbarium. The stromata (fig. 20) are flat or irregularly discoid, 1—4 mm wide, white, rough to slightly verrucose from the perithecia. The perithecia are flask-shaped, 450—500 × 250—300 μ. The asci are cylindric, 200—250 × 6—7 μ with 4 μ thick caps. The ascospores are filiform, nearly as long as the asci, 2 μ thick, multiseptate with cells 8—12 μ long. Part-spores were not found. Conidia are produced in the center of the stroma on an open hymenium or in an open ring between the border perithecia and a sterile center. They are fusoid, 8—10 × 1 μ with acute ends. The paraphyses are up to 100 μ long.

As previously stated PETCH has considered this as conspecific with *H. sloaneae* from which however it is distinguished by lack of tubercules, smaller asci and probably non-fragmenting ascospores. The open hymenium of the conidial stage is unusual. It may be due to the coalescing of crowded pycnidia.

Summary

Additional information is given from studies of types and authentic specimens for 25 species of Hypocrella. *Stereocrea aurantiaca* is transferred to Hypocrella as *H. aurantiaca* (PETCH) comb. nov., *H. caulium* is considered an invalid name and *H. brasiliana* (P. HENN.) comb. nov. is proposed. It is concluded that *H. sloaneae, H. nectroides, H. citrina, H. melaena, H. disjuncta* and *H. amazonica* all are valid species. *H. verruculosa* is considered conspecific with *H. phyllogena*. *H. fluminensis* is a synonym of *H. epiphylla* and *H. guaranitica, H. spegazzini, H. orbicularis* and *H. cornuta* are synonyms of *H. palmae*. Similarities between several species of the eastern and western hemispheres are noted.

Literature Cited

HENNINGS, P. (1897) Fungi camerunenses II. Bot. Jb. **23:** 536—558.

PATOUILLARD, N. (1914) Quelques champignons du Congo. Bull. Soc. mycol. Fr. **30:** 336—346.

PETCH, T. (1921) Studies in entomogenous fungi II. The genera Hypocrella and Aschersonia. Ann. Roy. Bot. Gard. Peradeniya **7:** 167—278.

— — (1925) Entomogenous fungi: additions and corrections. Trans. Brit. mycol. Soc. **10:** 190—201.

SACCARDO, P. A. & TROTTER, ALEX (1913) Ascomycetae-Deuteromycetae. Syll. fung. 22 sect. **1:** 1—822.

SPEGAZZINI, CARLO (1881) Fungi Argentini. An. Soc. cient. argent. **12:** 194—227.

SYDOW, H. & P. (1907) Verzeichnis der von Herrn F. Noack in Brasilien gesammelten Pilze: Ann. mycol. **5:** 348—363.

THAXTER, ROLAND (1914) Note on the ascosporic condition of the genus Aschersonia Montagne. Bot. Gaz. **57:** 308—313.

THEISSEN F. (1911) Die Hypocreaceen von Rio Grande do Sul, Südbrasilien. Ann. mycol. **9:** 40—73.

VIÉGAS, A. P. (1944) Alguns Fungos do Brasil II, Ascomicetos. Bragantia **4:** 5—392.

ZIMMERMANN, A. (1901) Einige javanische auf Cocciden parasitierende Ascomyceten. Zbl. Bakt. **7:** 872—876.

PENICILLIUM MARNEFFEI N. SP., AGENT D'UNE MYCOSE DU SYSTÈME RÉTICULO—ENDOTHÉLIAL

par

G. Segretain

Service de Mycologie et de Physiologie Végétale, Institut Pasteur Paris

(2.V.1959)

En mycologie médicale, les *Penicillium* isolés de produits pathologiques sont, à juste raison, considérés dans la plupart des cas, comme des champignons saprophytes contaminants. Cependant, il semble qu'ils puissent jouer un certain rôle pathogène dans des mycoses superficielles et en particulier dans des otomycoses du conduit auditif externe et dans des onychomycoses. *Scopulariopsis brevicaulis*, champignon voisin des *Penicillium* et portant des fructifications simplifiées, a été le plus souvent rencontré. Une excellente mise au point de la pathogénicité des *Scopulariopsis* a été donnée par Coutelen et coll. (6) lors de la description d'une espèce nouvelle de ce genre provenant de l'expectoration d'un malade.

Des *Penicillium* sont de temps en temps rencontrés dans des affections broncho-pulmonaires; ils doivent être considérés le plus souvent comme des saprophytes végétant sur des tissus lésés dans un organisme affaibli. Cependant leur rôle pathogène doit être envisagé dans de rares cas où les filaments mycéliens ont été trouvés dans les expectorations ou aspirations bronchoscopiques et où l'amélioration du malade a été obtenue par traitement antifongique. C'est le cas de *P. bertai* décrit par Talice & Mackinnon, et qui possède certains caractères d'*Aspergillus fumigatus* (17). C'est également celui de *P. crustaceum*, voisin de *P. glaucum*, rencontré par divers auteurs et en particulier par Aimé et coll. (1) qui malheureusement ne donnent aucun des caractères botaniques de leur champignon; celui-ci possèderait une certaine pathogénicité pour l'animal. Delore et coll. retrouvent par bronchoscopie d'un même malade, à deux ans d'intervalle, le même champignon, *P. spinulosum* dont quelques caractères seulement sont indiqués; des filaments mycéliens attribués au même champignon ont été trouvés chez ce malade dans le tissu musculaire d'une biopsie de nodule sous-cutané dont les cultures ont été négatives (7, 8).

D'autre part, *P. mycetogenum* est décrit comme agent d'un mycétome à grains noirs dont l'étiologie semble douteuse (12), et *P. citrinum* est isolé à l'état pur d'une urine où il forme des pelotons filamenteux, le rein droit étant seul atteint (11). Enfin, BRAUNSTEIN décrit dans sa thèse quatre cas de mycoses pulmonaires ou urinaires causées par des *Penicillium* sans description des agents pathogènes (4).

Dans une publication antérieure (5), nous décrivions avec CAPPONI et SUREAU la pathogénicité très particulière d'un *Penicillium,* agent d'une réticulose semblable à l'histoplasmose ou à la leishmaniose avec pullulation du parasite dans les cellules réticulo-endothéliales. Nous notions certains caractères particuliers de ce champignon que nous considérions comme une espèce nouvelle et que nous nommions *Penicillium marneffei* n. sp.

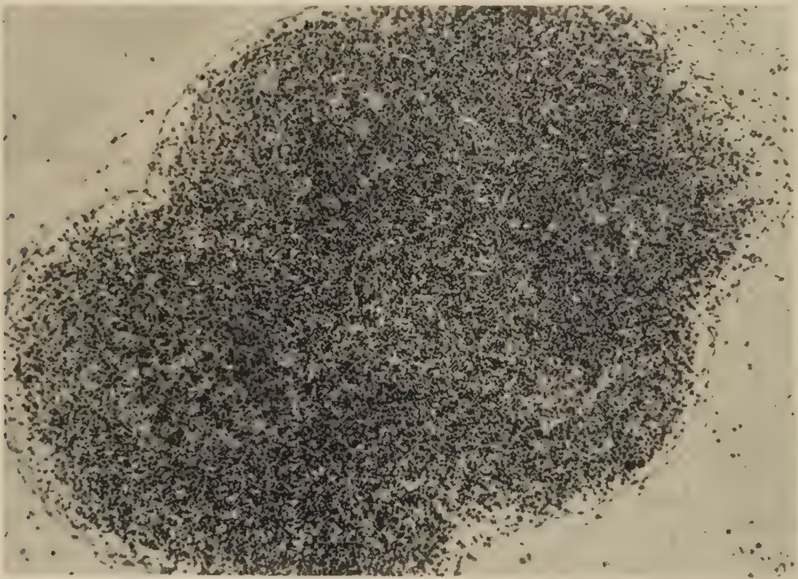


fig. 1. Nodule épiploique de *Rhizomys sinensis* parasité par place. Hématoxyline-éosine, × 68.

CAPPONI & SUREAU ont isolé *Penicillium marneffei* d'un rongeur des Hauts Plateaux du Centre Viet-Nam, le *Rhizomys sinensis,* spontanément infecté. Dans la note précédente, nous donnions la description suivante de la maladie spontanée et de l'isolement du parasite: ,,Deux de ces animaux, maintenus en captivité à l'Institut Pasteur de Dalat, et non inoculés, ont présenté de l'inappétence, sont devenus somnolents et ont été trouvés agonisants, sur le flanc, en hypothermie. On note à l'autopsie, un gros abdomen, une rate légèrement hypertrophiée, une ascite, des nodules épiploïques (fig. 1) et sur coupe, *une réticulose macrophagique de la rate, du foie,* *de l'épiploon et des poumons avec infiltration par des polynucléaires.* Dans les macrophages, de petites masses arrondies, rappellent des formes fongiques (fig. 2). Un troisième *Rhizomys* jeune, inoculé avec *Rickettsia orientalis* le 17-8-1955, meurt le 8-9-1955 présentant *gros foie, grosse rate* verruqueuse, ascite visqueuse et *nodules épiploïques.* L'ensemencement d'organes sur gélose Sabouraud glucosée à 25° donne une culture d'un *Penicillium* vert bleuté, rougissant rapidement le milieu de culture. Sur frottis, on ne retrouve pas les rickettsies".

En retrouvant le champignon après passage sur souris mortes 9 à 30 jours après inoculation, CAPPONI & SUREAU ont montré le rôle pathogène de ce *Penicillium.*

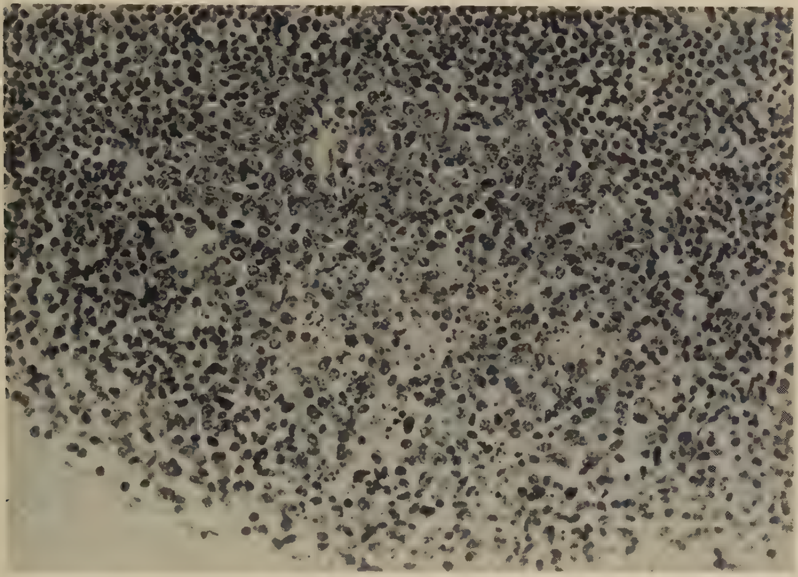


fig. 2. Plage du nodule précédant, formée de cellules histioplasmocytaires, contenant des granulations arrondies. Hématoxyline-éosine. × 260.

A Paris, nous avons étudié d'une part le pouvoir pathogène de *Penicillium marneffei* sur divers animaux de laboratoire et d'autre part les caractères botaniques et physiologiques du champignon. Nous rendons compte de ce travail dans ce mémoire.

I — POUVOIR PATHOGÈNE EXPÉRIMENTAL DE PENICILLIUM MARNEFFEI

P. marneffei a été inoculé à la souris, au lapin, au cobaye, au hamster et au rat; la sensibilité du champignon à des antibiotiques antifongiques a été étudiée; enfin, il est rendu compte de son action sur l'homme accidentellement inoculé.

A) Pouvoir pathogène pour la souris

Dans une première expérience, 10 souris sont inoculées par voie intrapéritonéale avec deux cultures sporulées du *Penicillium marneffei* reçues de Dalat, l'une isolée du *Rhizomys sinensis* (souche 1, 4 souris), l'autre isolée après passage sur souris (souche 2, 6 souris). Ces animaux sacrifiés 21 jours après l'inoculation, présentent à l'autopsie, un gros foie friable, une grosse rate, des ganglions inguinaux hypertrophiés, des micro-abcès sur le péritoine et un abcès au point d'inoculation. Les rétrocultures du foie, de la rate et des nodules sont positives.

Dans une deuxième expérience, 13 souris reçoivent chacune dans le péritoine, 0,3 ml de suspension d'arthrospores de *Penicillium marneffei* souche 1 (6 souris) et souche 2 (7 souris). Ces arthrospores viennent de cultures de 10 jours sur moût de bière gélosé à 37°. Les animaux sont sacrifiés à des temps variables: 4 après 11 jours et quatre après 21 jours (chaque fois, deux souris inoculées avec la souche 1 et deux avec la souche 2), enfin les cinq animaux restant, après 6 mois. Les huit premières souris présentent à l'autopsie les caractères suivants plus ou moins marqués: liquide péritonéal, gros foie, grosse rate, inflammation des ganglions inguinaux et présence de nodules dans la cavité péritonéale. Toutes les rétrocultures de foie, de rate et de nodules sont positives bien que quelques animaux présentent beaucoup moins de lésions que d'autres. A l'autopsie de 5 animaux sacrifiés après 6 mois, on constate que les lésions ont regressé et, si d'une façon constante il y a un abcès au point d'inoculation, un seul animal présente un gros foie et une grosse rate, et un autre une orchite purulente.

Les deux souches ayant la même pathogénicité vis à vis de la souris et, comme nous le verrons plus loin vis à vis du hamster, une seule des deux souches (souche 2) sera inoculée ultérieurement. Mais dans ces deux expériences, nous n'obtenons aucune mortalité chez cet animal, alors que les souris inoculées à Dalat par CAPPONI & SUREAU mourraient pour la plupart 15 jours à 1 mois et rarement plus de deux mois, après l'inoculation. Ces auteurs notaient dans un cas (mortalité 9 jours après l'inoculation), une localisation rénale que nous n'avons jamais observée et dans la plupart des cas, une splénomégalie beaucoup plus considérable que celle que nous avons obtenue chez nos animaux. Chez une souris envoyée par avion de Dalat à Paris et morte spontanément, la rate et le foie étaient considérablement hypertrophiés et ce dernier organe de texture friable et granuleuse avait une surface grillagée.

La faible sensibilité de nos souris à l'inoculation de *Penicillium marneffei* pouvait être un caractère dû à la lignée élevée au laboratoire. Aussi, dans l'expérience suivante, 3 lignées différentes de souris sont inoculées: souris du laboratoire, souris suisses et souris KR 398. Huit souris dans chaque lot reçoivent chacune une injection intrapéritonéale de 0,3 ml d'une suspension d'arthrospores et de filaments provenant de cultures de 3 jours à 37°, sur milieu à l'œuf de Kurung. Un mélange de pénicilline-streptomycine est ajouté à la suspension pour éviter les infections bactériennes. Deux souris de chaque lot sont sacrifiées 7, 15, 25 jours et 6 mois après l'inoculation. La sensibilité de ces trois lots de souris s'est montrée à peu près la même: aucune des souris n'est morte spontanément de pénicillose expérimentale et lors des autopsies, à côté des lésions classiques, abcès au point d'inoculation, gros foie, grosse rate et nodules dans la cavité péritonéale, on peut noter une sensibilité péritonéale plus importante de la lignée KR 398, des nodules ou abcès plus gros avec les souris suisses, et la présence presque constante de liquide péritonéal avec les souris du laboratoire. Les animaux sacrifiés 6 mois après l'inoculation, montrent une tendance vers la guérison: les rétro-cultures de foie et de rate sont négatives à une exception près (souris KR 398), mais le champignon est encore vivant dans des abcès du testicule chez les 2 souris du laboratoire et dans des ulcères intestinaux des souris suisses.

Nous pensons donc que la grande sensibilité des souris de Dalat à *Penicillium marneffei* n'est probablement pas dûe à une lignée particulière, mais, soit à une nourriture légèrement déficiente, soit plutôt à un affaiblissement du pouvoir pathogène de la souche que nous constaterons ultérieurement.

Le passage sur souris n'augmente pas le pouvoir pathogène de la souche. L'inoculation intra-péritonéale à 11 souris, de broyats de foie ou de nodules, ou bien de liquide péritonéal de souris sacrifiées, n'amène pas de mortalité spontanée chez ces animaux. Il en est de même de l'inoculation intra-veineuse: sur les 6 souris sacrifiées deux mois plus tard, on ne trouve aucune lésion chez deux mâles, et les rétro-cultures d'organes sont négatives; chez les 4 femelles, malgré de grosses rates et des nodules sur les ovaires ou leurs annexes, les rétro-cultures de foies avec abcès sont seules positives.

B) Pouvoir pathogène pour le lapin et le cobaye

Deux lapins et quatre cobayes se sont montrés insensibles à l'inoculation d'une suspension d'arthrospores du champignon obtenues par culture sur moût de bière gélosé à 37°. Les lapins ont reçu 2 ml de suspension par voie intraveineuse et les cobayes des injections soit intradermique, soit intra-musculaire, soit intra-péritonéale (1 ml de suspension). Aucune lésion n'a été notée à l'autopsie des animaux sacrifiés aussi bien que sur coupes d'organes fixés. Cependant, à Dalat, Capponi & Sureau obtenaient la mort de cobayes 20 jours après l'inoculation sous-cutanée et 11 jours après l'inoculation intra-testiculaire de broyat de rate de souris parasitée.

C) Pouvoir pathogène pour le hamster et le rat

Lors de nos expériences à Paris, les deux animaux qui se sont montrés les plus sensibles à l'inoculation de *Penicillium marneffei* sont le hamster et le rat.

1 — *Hamster.*

Quatre hamsters inoculés par voie intrapéritonéale avec une suspension d'arthrospores du champignon obtenues à 37° sur moût de bière gélosé, sont morts spontanément 30, 32, 43 et 49 jours plus tard. A l'autopsie, les poumons sont légèrement hémorragiques, le foie très hypertrophié a une surface grillagée et la rate très allongée, fortement épaissie, déformée par des bosses, peut être plus ou moins décolorée par place. Chez le premier hamster mort, des ulcères localisés sont visibles sur l'intestin; quelques jours avant sa mort, cet animal avait le ventre humide, ses crottes transparentes, mises en culture après récolte aseptique ont donné d'assez nombreuses colonies de *Penicillium.* L'inoculation intrapéritonéale d'un broyat de foie de ce hamster chez deux hamsters neufs, a provoqué leur mort 20 jours plus tard; leurs organes présentent à l'autopsie les mêmes lésions que les précédents. Après avoir montré rapidement de l'inappétence puis de la somnolence, ces six animaux sont morts cachectiques.

Dans une autre expérience réalisée au cours d'essai de traitement et dont nous rendons compte plus loin, 5 hamsters inoculés dans les mêmes conditions sont morts 13 ou 14 jours après l'inoculation.

2 — *Rat.*

Deux rats sont inoculés par voie intra-veineuse, recevant chacun 1 ml d'une suspension d'arthrospores. Ils meurent 14 et 19 jours plus tard. A l'autopsie, le foie est gros, grillagé et entièrement de couleur jaune d'or; la rate très grossie, présente des plages jaune d'or et est grillagée par place; enfin, les poumons sont légèrement hémorragiques (fig. 3). Une suspension d'un broyat de rate de hamster parasitée, inoculée au rat par voie intrapéritonéale a provoqué la mort de l'animal 20 jours plus tard.

3 — *Souches atténuées.*

Cependant, cinq mois plus tard, 8 rats inoculés dans le péritoine avec un broyat de rate de hamster parasitée ne sont pas morts spontanément. Le foie et la rate des animaux sacrifiés un mois ou deux mois après l'inoculation ne présentent que quelques nodules blanchâtres plus ou moins nombreux. Les rétro-cultures de rate sont cependant toutes positives et deux cultures de foie d'aspect presque normal sont seules restées négatives.

L'aspect des cultures de *P. marneffei* s'étant modifié, devenant presque entièrement laineuse et de couleur blanc rosé sans sporulation, nous avons réisolé des souches du champignon, soit à partir

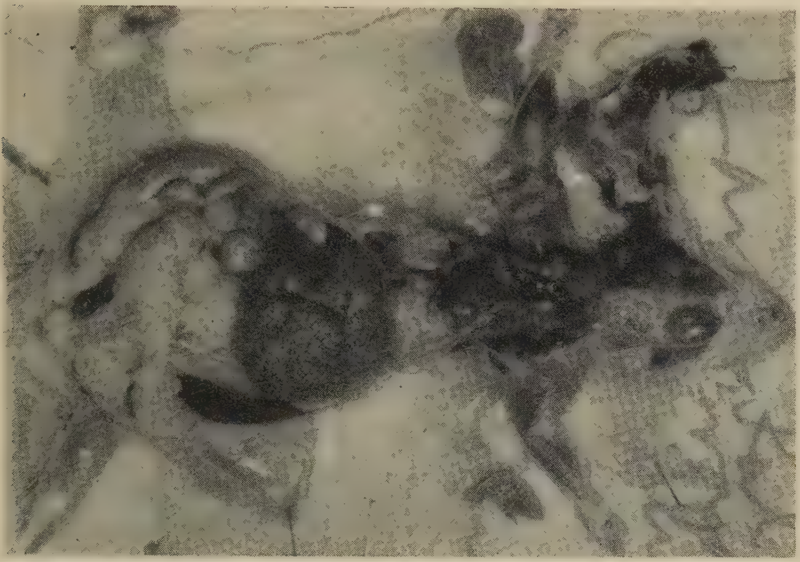


fig. 3. Hypertrophie du foie et de la rate chez un rat mort 14 jours après l'inoculation.

de spores, soit à partir d'arthrospores et étudié leur pouvoir pathogène sur le hamster. Une suspension de spores obtenues par culture sur milieu de Czapek à la température du laboratoire et une suspension d'arthrospores obtenues par culture sur moût de bière gélosé à 37°, sont ensemencées en stries sur différents milieux: moût de bière gélosé, Sabouraud glucosé, Sabouraud maltosé, milieu de Kurung. Cinq jours plus tard, on isole trois colonies séparées, deux à partir d'arthrospores, et une à partir de spores. Après culture sur moût de bière à 37°, des suspensions d'arthrospores de chacune des souches sont inoculées dans le péritoine du hamster à raison de deux ou trois animaux par souche. Le foie et la rate des animaux sacrifiés un mois plus tard ne présentent pour la plupart aucune lésion. Quelques nodules sont parfois visibles dans la cavité péritonéale, les rétro-cultures de rate et de nodules sont toutes positives et celles du foie dans trois cas sur sept. Cependant, l'un des hamsters inoculé avec la souche isolée de la suspension de spores est nettement plus atteint, la rate est grosse et déformée, le péritoine couvert de micro-nodules, l'épiploon est hémorragique et épaissi. Le broyat de la rate de ce hamster est inoculé dans le péritoine de 4 hamsters neufs, à raison de 0,5 à 1 ml par animal. Les animaux sacrifiés 18 et 35 jours plus tard présentent des reactions nodulaires nettement plus importantes dans la cavité péritonéale et sur la rate et le foie qui présente des aires grisâtres en surface et jaune verdâtre sur la coupe. Toutes les rétrocultures sont positives.

Il semble donc possible de redonner de la virulence à une souche atténuée de *P. marneffei* en réisolant le champignon à partir de spores puis par passage sur le hamster.

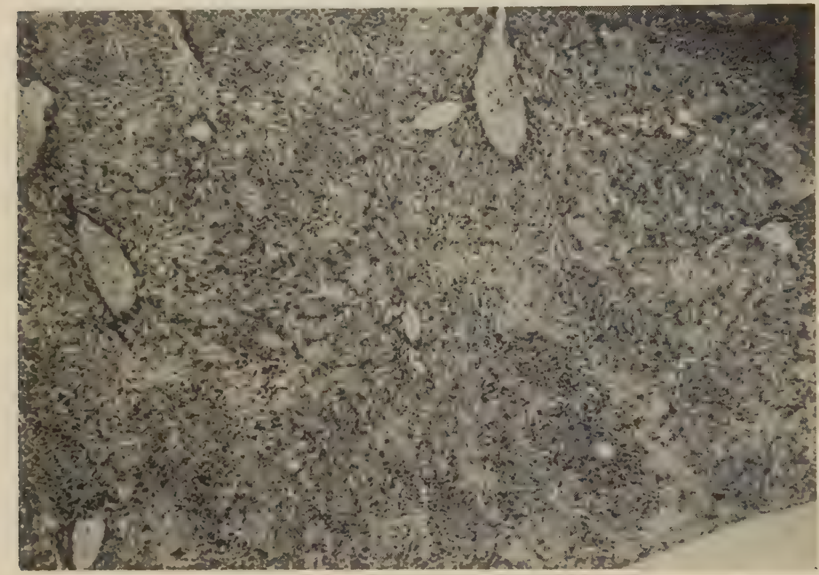


fig. 4. Envahissement du foie de hamster par les cellules réticulaires. Hématoxyline-éosine. × 31,5.

D) Histopathologie des lésions

L'étude histologique des organes des animaux inoculés avec *Penicillium marneffei* révèle le caractère particulier de cette pénicillose: la lésion est en effet une réticulose macrophagique diffuse de la rate, du foie et même du poumon, semblable à celle de l'histoplasmose et de la leishmaniose. Chez les animaux sensibles qui meurent spontanément après l'inoculation, le protoplasme des cellules histio-réticulaires est occupé en totalité par les parasites (fig. 10A).

Nous avons trouvé cette réticulose à l'état pur dans le foie très hypertrophié d'une souris inoculée à Dalat, expédiée à Paris et morte peu après son arrivée. La réticulose de l'organe, ne respectait que des éléments du parenchyme hépatique fonctionnel restés indifférents. Les autres organes de l'animal ne semblaient pas parasités.

Dans le foie des hamsters et des rats morts spontanément, la réticulose reste plus localisée autour des espaces portes (fig. 4). Au voisinage des lésions, le parenchyme hépatique est profondément altéré par la nécrose fibrinoïde et l'oedème (fig. 5). Chez le rat, le parasite possède en outre une nette action toxique vis à vis des cellules qu'il envahit: les noyaux se déforment, se vacuolisent et disparaissent. La rate et les nodules lymphatiques de ces deux animaux sont le siège d'une réticulose diffuse et massive et ont perdu toute structure. Des histiocytes parasités sont visibles dans les travées pulmonaires légèrement épaissies, dans les glomérules du rein fortement congestionnés, dans les méninges et les vaisseaux du cerveau par ailleurs intacts.

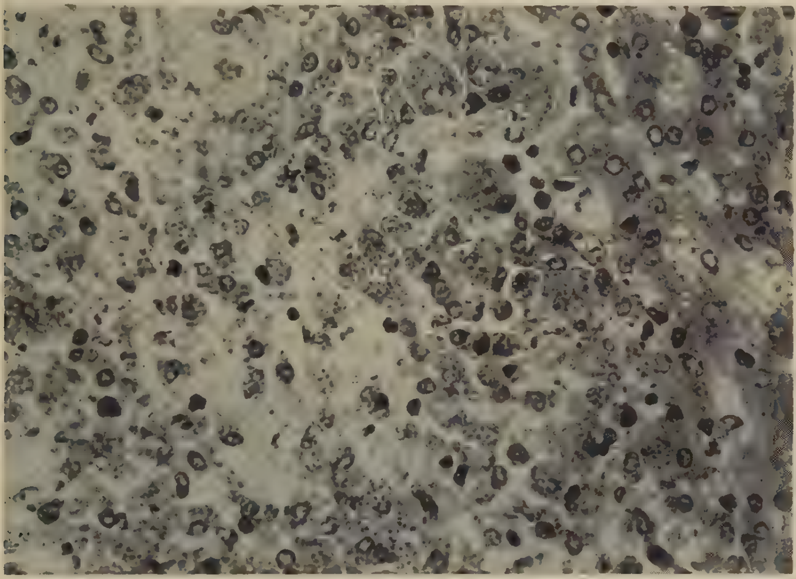


fig. 5. Les cellules réticulaires, dans l'intérieur desquelles les parasites sont visibles, envahissent le tissu hépatique altéré. Hématoxyline-éosine. × 350.

Les lésions intestinales trouvées chez le hamster prennent naissance dans les plaques de Peyer; puis l'inflammation gagnant en largeur et en profondeur, elles prennent la forme de coin ou de „bouton de chemise'' (fig. 6). La muqueuse intestinale est traversée, ce qui explique que le champignon ait été retrouvé dans les crottes de l'un des animaux quelques jours avant sa mort. Les abcès de la muqueuse intestinale sont nécrotiques et les cellules parasitées ne sont visibles qu'à leur périphérie.

Chez toutes nos souris en expérience ainsi que chez les hamsters inoculés avec le champignon ayant perdu de sa virulence et ne provoquant plus la mort des animaux, les lésions du foie et de la rate ainsi que les nodules de la cavité péritonéale, sont nettement

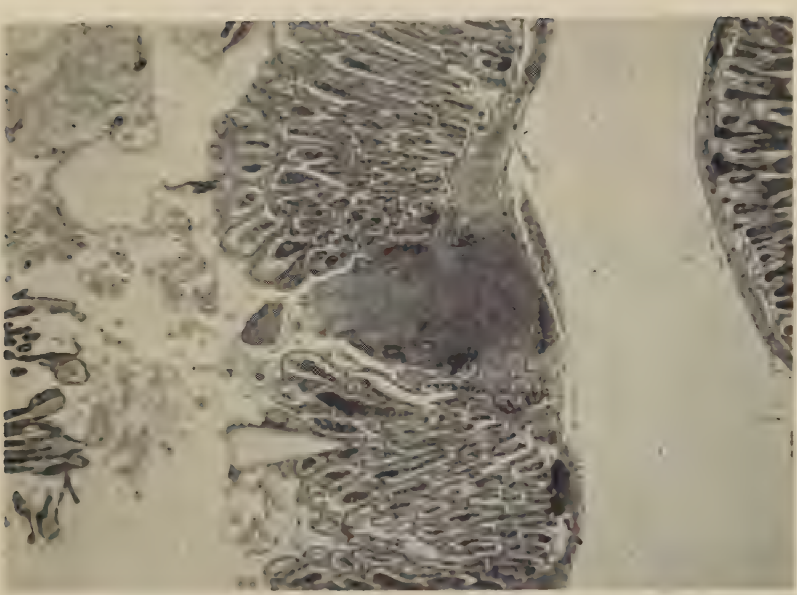


fig. 6. Lésion nécrosée en forme de "bouton de chemise" de la muqueuse intestinale du hamster. Hématoxyline-éosine. × 23.

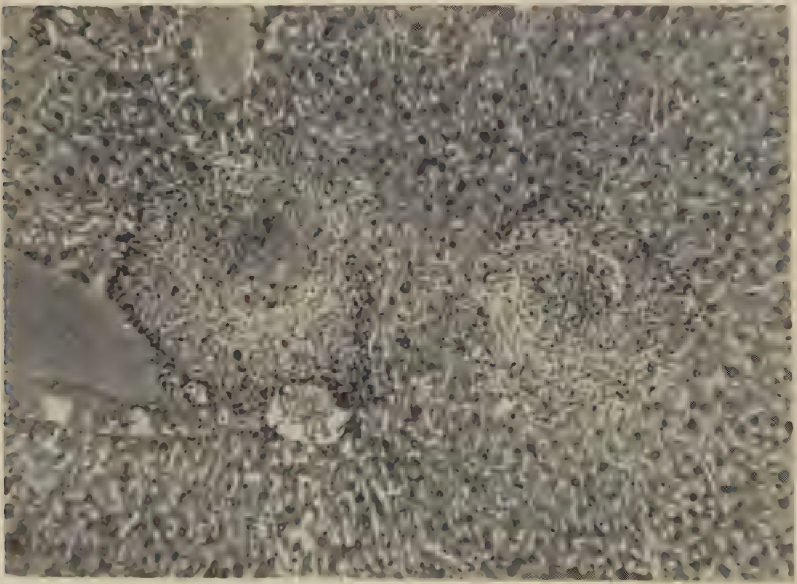


fig. 7. Deux lésions bien délimitées d'un foie de souris; l'une jeune, l'autre plus agée et nécrotique en son centre. Hématoxyline-éosine. × 85.

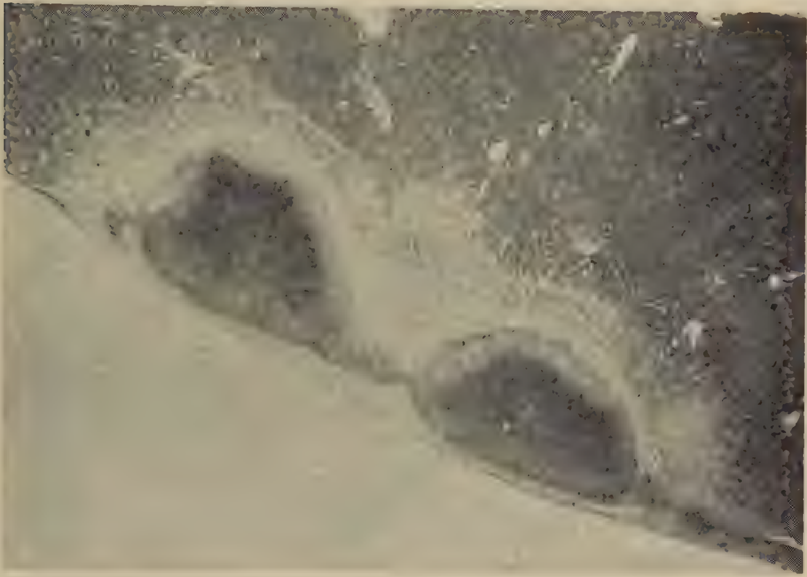


fig. 8. Lésions périhépatiques de la souris, entièrement nécrosées au centre. Hématoxyline-éosine. × 36,5.

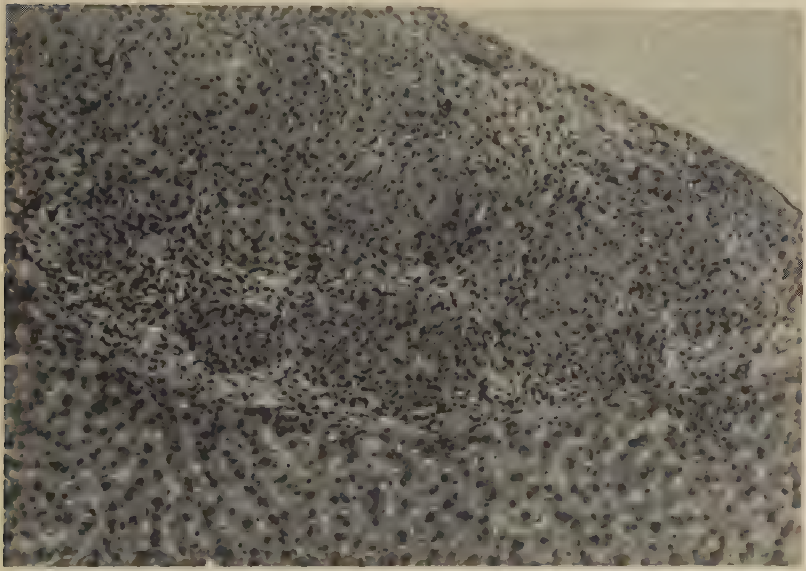


fig. 9. Lésion nodulaire périhépatique du hamster, provoquée par une souche atténuée de *P. marneffei*. Hématoxyline-éosine. × 135.

délimités sans tendance à l'envahissement. La lésion jeune formée de cellules réticulaires parasitées, se nécrose rapidement (fig. 7): la zone centrale formée de restes de noyaux en pycnose, est entourée d'histiocytes et de monocytes non parasités, mêlés à quelques lymphocytes (fig. 8). Dans certaines lésions de périhépatite du hamster (fig. 9), de rares grosses formes parasitaires sont visibles dans les histiocytes.

E) Sensibilité de Penicillium marneffei à la nystatine et à l'actidione in vitro et in vivo.

E. Drouhet, qui a effectué ce travail, trouve d'abord que *Penicillium marneffei* est sensible *in vitro* à la nystatine et à l'actidione: la nystatine inhibe la croissance en milieu liquide semi-synthétique après 24 heures à une dose inférieure à 0,65 μg/ml et après 4 jours à la dose de 3,2 μg/ml, l'actidione agit de même, inhibant la croissance du champignon en milieu de Sabouraud gélosé à une dose comprise entre 3,1 et 6,25 μg/ml après 48 heures.

Le champignon étant sensible *in vitro* à ces antifongiques, on a recherché leur action dans la pénicillose expérimentale du hamster et de la souris.

Hamsters.

14 hamsters jeunes (30 g) reçoivent chacun par voie intrapéritonéale, 0,25 ml d'une suspension d'un broyat de foie de hamster parasité. Sur les six témoins sans traitement, 5 meurent spontanément 13 jours ou 14 jours après l'inoculation. Le 6 ème est sacrifié après 20 jours. Tous présentent une ascite, un foie énorme, une rate modérément hypertrophiée et des foyers congestifs pulmonaires plus ou moins visibles; toutes les rétrocultures effectuées sont positives. Huit hamsters sont traités pendant 18 jours deux fois par jour, par injections sous-cutanées de chacune 0,5 mg d'antifongique: nystatine ou actidione. Ceux traités à la nystatine sont encore vivants 20 jours après l'inoculation; ils sont alors sacrifiés et on ne note à l'autopsie qu'une légère hypertrophie du foie et de la rate. Sur les quatre hamsters traités à l'actidione, deux meurent le 4ème jour, ce qui indique la toxicité du produit, et les deux autres le 11ème jour; tous les organes mis en culture de ces 4 animaux donnent de nombreuses colonies de *Penicillium.*

Souris.

17 souris reçoivent dans le péritoine 0,5 ml d'une suspension d'un broyat du foie d'un hamster parasité. Cinq animaux, témoins non traités, sacrifiés 21 jours plus tard, présentent une hypertrophie du foie et de la rate, des nodules péritonéaux et un abcès au point d'inoculation; les rétrocultures d'organes sont toutes positives. Six animaux sont traités à la nystatine: ils reçoivent pendant les

14 premiers jours deux fois par jour, 0,5 mg de nystatine par injection sous-cutanée, puis pendant trois jours, dans les mêmes conditions, 1 mg de nystatine par injection. A l'autopsie des animaux sacrifiés 21 jours après l'inoculation, les organes de trois d'entre eux paraissent sains; chez les trois autres, le foie est hypertrophié avec abcès chez un seul animal, deux rates sont grosses et une seule souris la plus atteinte présente des nodules péritonéaux. Chez ce dernier animal, les rétrocultures de rate et de foie se développent comme chez les témoins; elles restent négatives chez un des hamsters sans lésions apparentes. Chez les quatre autre les cultures apparaissent tardivement et les colonies sont rares. Les six souris traitées pendant 17 jours à l'actidione, à raison de 1 mg par jour en deux injections sous-cutanées, et sacrifiées après 21 jours, présentent à l'autopsie, le même aspect que les témoins, et les rétrocultures d'organes se développent de façon identique à celles des témoins.

La nystatine par voie sous-cutanée se montre donc très efficace sur la pénicillose expérimentale du hamster et de la souris; l'actidione est sans action et montre une certaine toxicité pour le hamster.

F) Pathogenicité du Champignon pour l'Homme

Au cours de l'inoculation de rats par voie intraveineuse, nous nous sommes piqué accidentellement à l'index droit avec l'aiguille ayant servi à inoculer les deux premiers animaux; la sensation est nette sans apparition de goutte de sang. Neuf jours plus tard un petit nodule apparait au point d'inoculation, et, au niveau du biceps, un cordon lymphatique sur lequel se formeront deux petits nodules en grains de plomb; un ganglion légèrement hypertrophié apparaîtra quelques jours plus tard à l'aisselle.

Le nodule de l'index dont le diamètre est de 3 mm environ, est prélevé 12 jours après l'inoculation et *P. marneffei* est retrouvé dans la culture du nodule broyé au mortier. La ponction ganglionnaire est négative par culture et examen des frottis. Le champignon étant sensible à la nystatine, un traitement par cet antibiotique antifongique est commencé à raison de 40 dragées glutinisées par jour (20 millions d'unités); il sera poursuivi pendant un mois. La réaction ganglionnaire s'efface rapidement, mais le cordon lymphatique et ses petits nodules persisteront pendant plus d'un mois.

Cinq jours après l'apparition du nodule de l'index, une intradermoréaction avec un filtrat non dilué de culture du champignon a été effectuée chez l'intéressé et chez un sujet sain. Une forte infiltration nodulaire avec érythème est apparue le lendemain chez les deux sujets, a atteint 10 cm de diamètre et ne s'est calmée que 4 jours plus tard. Elle indique la forte toxicité du filtrat de cultures pour l'homme.

G) Conclusion

Capponi & Sureau avaient provoqué à Dalat (Sud Viet Nam) la mort des souris inoculées par *Penicillium marneffei.* A Paris, malgré de nombreux essais, aucune mortalité n'a été obtenue chez cet animal, mais le hamster et le rat se sont montrés beaucoup plus sensibles comme en rend compte le tableau suivant:

Tableau I.

Maladie expérimentale à Penicillium marneffei

Nature de l'inoculum	Mortalité (en jours)	
	Hamster	Rat
Suspension d'arthrospores	30 à 50 inoculation par voie intrapéritonéale	14 à 19 inoculation par voie intraveineuse
Suspension de foie ou de rate de hamster broyé	13 à 20 inoculation par voie intrapéritonéale	13 à 20 inoculation par voie intrapéritonéale

La maladie est caractérisée par une réticulose macrophagique diffuse, tout à fait analogue à l'histoplasmose. Ces résultats confirment que le hamster est l'animal de choix pour l'étude expérimentale des mycoses profondes (13, 16) et en particulier de celles qui, comme l'histoplasmose, lèsent le système réticulo-endothélial (10). C'est un des caractères très particulier de *Penicillium marneffei.*

II — CARACTÈRES BOTANIQUES ET PHYSIOLOGIQUES DE PENICILLIUM MARNEFFEI.

A) Formes parasitaires de Pencillium marneffei.

Sur coupe histologique, le parasite est bien mis en évidence par coloration à l'hématéine-éosine ou au Giemsa; à l'Hotchkiss-Mac Mannus, il ne prend que faiblement la coloration rouge. Sur apposition d'organe, cette dernière technique de coloration teinte légèrement en rose la membrane, tandis que le May-Grünwald-Giemsa fait apparaître dans le parasite plusieurs granulations plus ou moins intensément colorées en violet. Sur frottis d'organe, nous n'avons jamais pû voir nettement le champignon, alors que sur apposition d'organe il est très visible; nous pensons que le parasite pourrait être très fragile et détruit par le frottis.

La morphologie de *Penicillium marneffei* et son mode de multiplication dans l'organisme animal parasité sont sans doute les caractères qui distinguent le plus ce parasite non seulement des

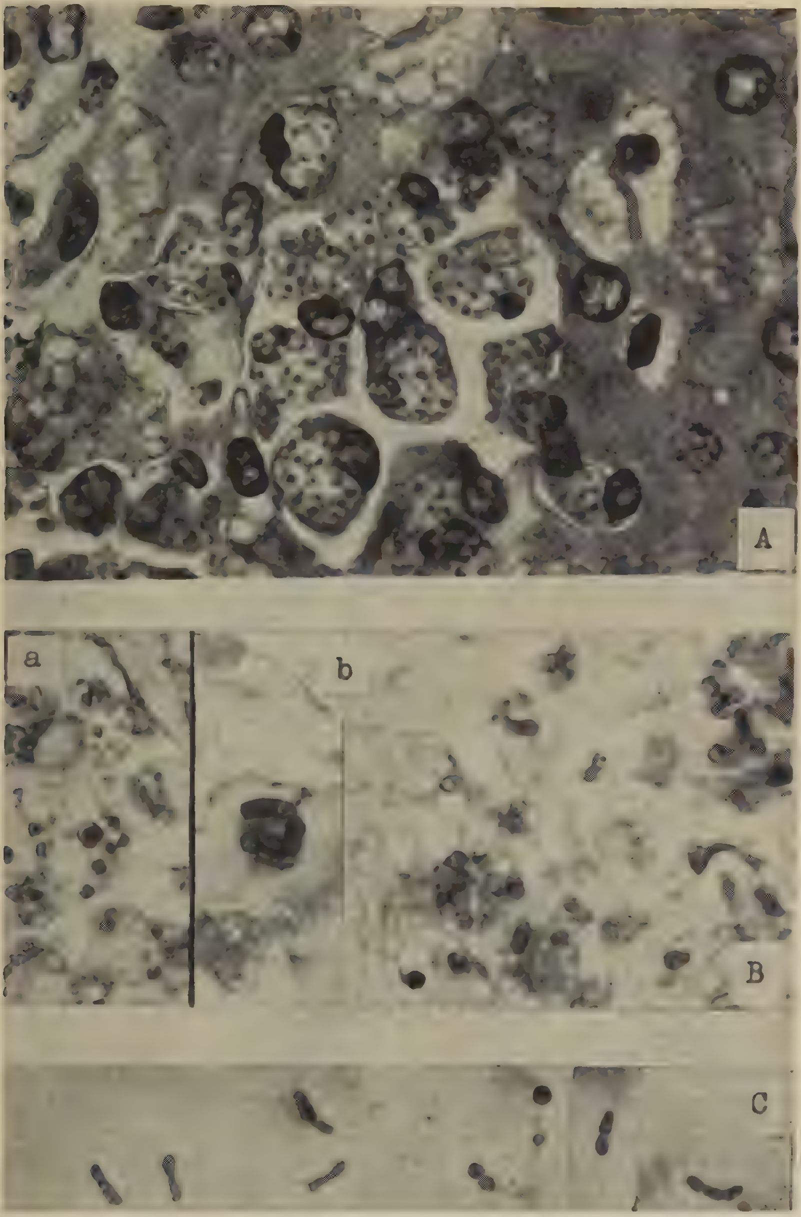


fig. 10A. Petites formes parasitaires incluses dans des cellules histioréticulaires d'un foie de hamster. Hématoxyline-éosine. × 1000.
fig. 10B. Formes parasitaires plus grosses et sphériques (a, rate de hamster) ou allongées et cloisonnées (b, rate de rat). Hotchkiss-Mac Mannus. × 1000.
fig. 10C. Formes semblables obtenues en culture sur moût de bière à 37°. Bleu coton au lactophénol. × 1000.

autres *Penicillium* mais encore des autres champignons pathogènes connus.

Sa forme est sphérique ou légèrement ovalaire et à première vue elle rappelle celle d'un histoplasme. A tel point que Capponi & Sureau nous ayant adressé de Dalat, lors d'un premier envoi, des frottis d'organes et la culture du *Penicillium,* nous leur avons répondu: histoplasmose ou leishmaniose et culture d'un saprophyte.

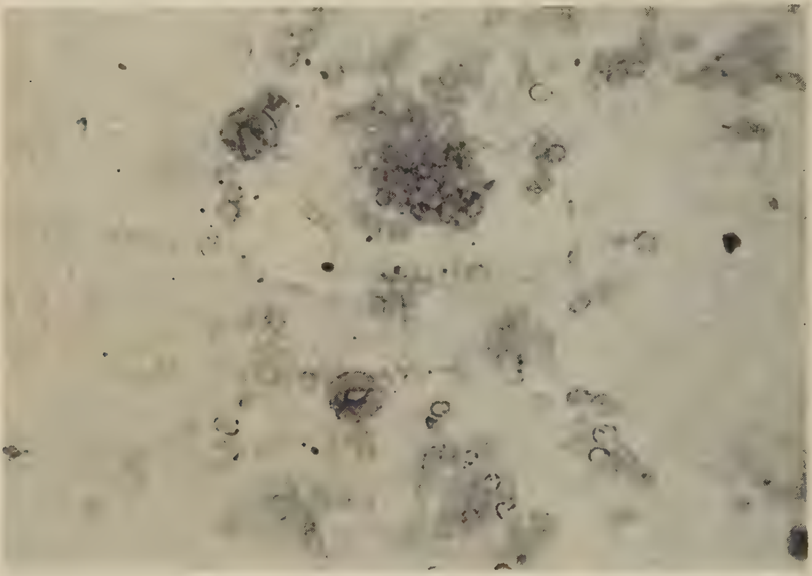


fig. 11. Frottis de foie de hamster. Formes arrondies ou ovalaires et courts filaments arqués et cloisonnés du parasite. Hotchkiss-Mac Mannus. × 930.

En effet, sur coupe, le parasite se présente comme un granule assez bien coloré, entouré d'un halo clair (fig. 10A) et sur coupe d'organe de hamster, quelques parasites nettement plus gros rappellent les formes plus grandes de l'histoplasmose à petite forme, signalées par de Monbreun, rencontrées chez le hamster et décrites par Drouhet & Schwarz (fig. 10B, a) (9). Mais les dimensions du parasite sont nettement supérieures à celles de l'histoplasme: elles varient entre 2 et 4,5 μ sur application d'organe colorée par la technique de Hotchkiss-Mac Mannus qui souligne légèrement en rose la membrane externe du champignon. On rencontre assez souvent des formes de même diamètre mais plus allongées et rarement de courts éléments filamenteux de 10 à 15 μ de long souvent plus ou moins arqués en forme de fer à cheval (fig. 11).

Le mode de multiplication du parasite *in vivo* est un des caractères les plus curieux de *Penicillium marneffei.* Nous n'avons en effet jamais rencontré de forme bourgeonnante du champignon, mais nous avons pû fréquemment voir à l'Hotchkiss une cloison tranver-

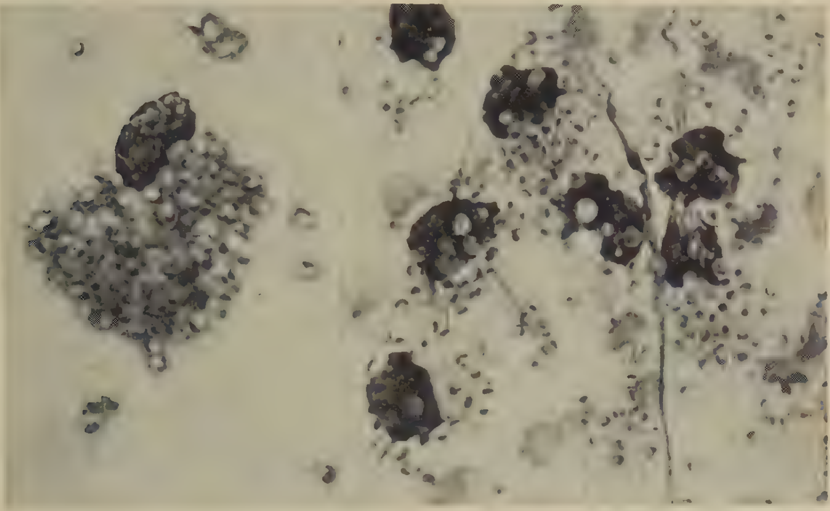


fig. 12. Frottis de foie de hamster coloré au May Grünwald-Giemsa × 670.

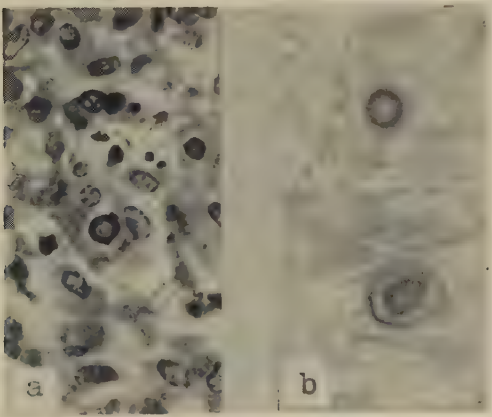


fig. 13. Grosses formes du parasite dans des lésions nodulaires du hamster. a) Hématoxyline-éosine. × 450. b) Hotchkiss-Mac Mannus. × 1000.

sale sur des éléments arrondis ou plus ou moins allongés et très rarement deux cloisons (fig. 10B, b); au May-Grünwald-Giemsa la membrane non colorée apparaît comme une solution de continuité bien dessinée (fig. 12). Ce parasite se multiplie donc *in vivo* par scissiparité.

De rares formes rondes d'un diamètre plus grand et pouvant atteindre 8 à 10 μ de diamètre se rencontrent au milieu des petites formes sur coupe de rate de hamster colorée par la technique de Hotchkiss-Mac Mannus. Ce sont les seules formes visibles chez le hamster dans les lésions de type nodulaire produites par le *Penicillium* qui a perdu son pouvoir pathogène. L'hémalun colore légèrement en violet la partie centrale de ces grandes formes tandis que la périphérie est formée d'enveloppes rouges (en forme de bulbe d'oignon) comme si le parasite de taille normale était entouré d'une capsule (fig. 13a). Mais à l'Hotchkiss le parasite semble entouré d'une membrane fortement colorée en rouge et à double contour (fig. 13b).

Enfin, il faut noter qu'aucune forme filamenteuse longue, caractéristique de la forme parasitaire des Aspergillacées n'a été vue chez *Penicillium marneffei.*

B) Caractères botaniques de Penicillium marneffei.

La croissance de *Penicillium marneffei* mesurée par le diamètre de ses colonies sur milieux gélosés est plus rapide à la température de 27°—30° qu'à la température du laboratoire à 22°; elle est retardée par la température de 37° (tableau) II.

TABLEAU II.

Age des cultures	Diamètre des cultures de *P. marneffei* (en cm)							
	Czapek			Sabouraud glucosé			Moût de Bière	
	22°	30°	37°	22°	30°	37°	30°	37°
3 jours	0,8	0,8	0,5	0,5	1,1	0,7	1,1	0,5
8 jours	1	2,4	0,8	2	—	1,2	3 (6 j.)	1,1 (6 j.)
16 jours	2	3	1,3	3,5	> 6cm	2	6 (13 j.)	2,1 (13 j.)

Sur milieu de Czapek, le champignon se développe à l'intérieur de la gélose et ce n'est que très tardivement qu'un léger feutrage sporulé apparait en surface. C'est pour cette raison que nous décrirons l'aspect des colonies et la morphologie du champignon sur moût de bière gélosé et sur Sabouraud glucosé, qui sont les milieux favorables à la fois au développement et à la fructification du champignon.

1°) *Moût de bière*

A la température de 27°, trois jours après l'ensemencement sur moût de bière gélosé, la colonie est plate, membraneuse, mate, finement plissée et de teinte blanc-brunâtre; le revers est incolore.

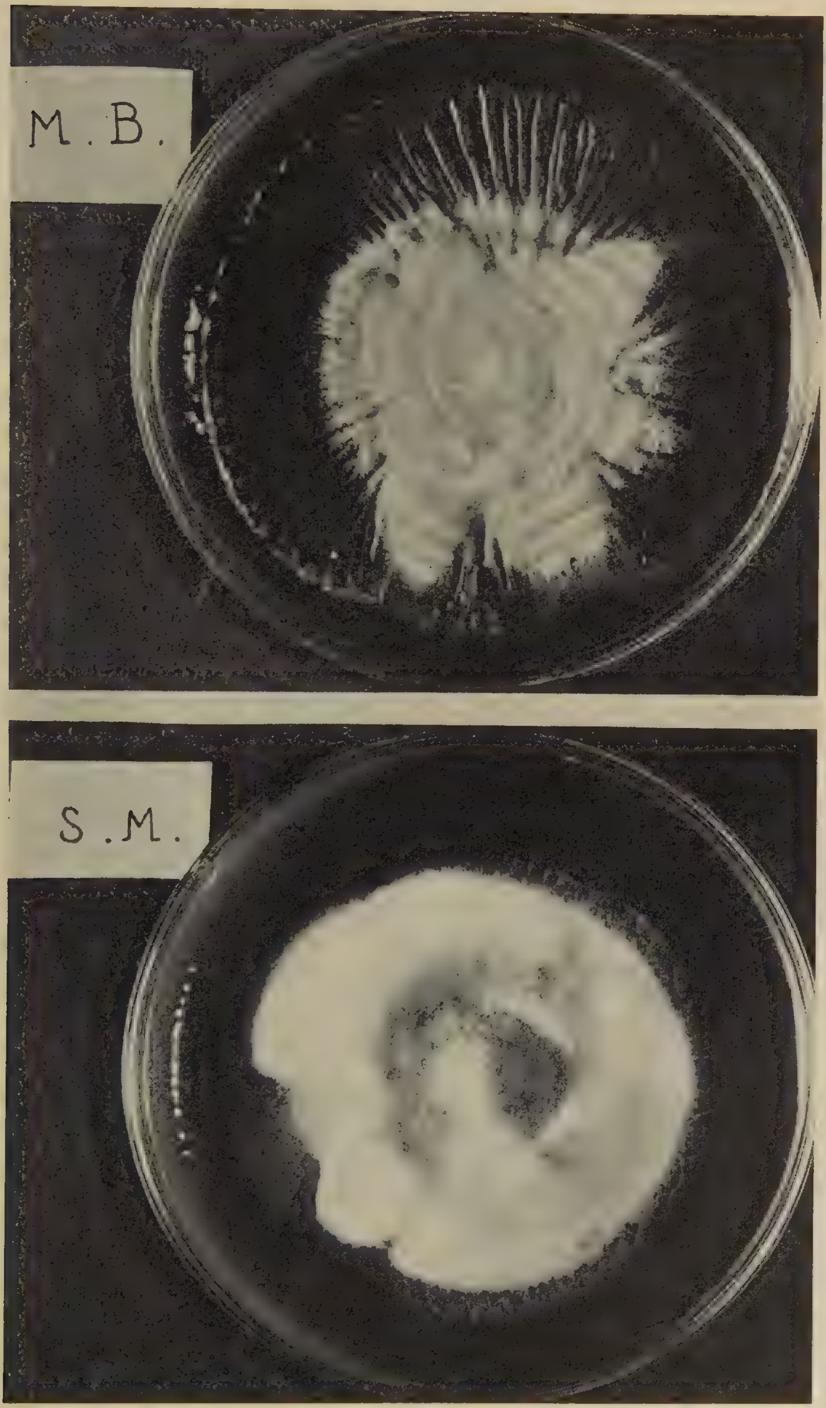


fig. **14**. Cultures de 15 jours à 30° de *Penicillium marneffei*.
M.B.) sur moût de bière gélosé. S.M.) sur gélose de Sabouraud maltosée.

Huit jours après l'ensemencement, le diamètre de la colonie est de 3,7 cm. A part une mince marge membraneuse, la culture est recouverte d'un duvet plat, vert gris bleuté (bleu d'horizon n° 3) (14) au centre et blanc à la périphérie; le revers et le milieu sont rouge brique (terre de Sienne brulée n° 1 à 2) par diffusion d'un pigment; cette coloration peut apparaître beaucoup plus rapidement, en 24 à 48 heures. La diffusion du pigment dans le duvet peut le teinter de rose par place (rose fleur de pêcher n° 1).

Après 17 jours (fig. 14a), la gélose du tube de culture est couverte d'une colonie plate, laineuse, gris vert bleuté assez clair (bleu turquoise n° 1), qui peut être zonée de rouge brique; la culture forme des plis visibles au fond du tube. Le revers est orange brun foncé et le milieu entièrement orange (terre de Sienne brulée n° 3 à 4). En vieillissant, la culture prend une teinte vert brun rosé plus ou moins lavée de blanc (vert glauque n° 2 ou 3), le revers restant rouge.

A la température de 30°, après 6 jours de culture, la colonie, qui dépasse 3 cm de diamètre et est restée glabre et ma, te se couvre souvent de mèches en forme de virgules dont les courbures sont toutes orientées dans le même sens; ceci donne à la colonie un aspect tournant en général dans le sens inverse des aiguilles d'une montre. Puis, en 24 ou 48 heures, la colonie se couvre de filaments aériens fuctifères, venant pour la plupart des mèches.

La surface laineuse de la colonie est formée de filaments entremêlés, cloisonnés, fins, d'1 à 2μ de diamètre. Les conidiophores sinueux prennent naissance sur les filaments aériens dont ils sont peu distincts; ils sont lisses, cloisonnés, et leur diamètre est en moyenne de 1,5 à 2μ; leur longueur très variable, de 70 à 175μ, est en moyenne de 111μ.

Le pinceau nettement divariqué, (fig. 15) est le plus souvent presque symétrique, et seul un examen attentif à fort grossissement, montre que les métules ne prennent pas toutes naissance à l'extrêmité du conidiophore. La longueur du pinceau est en moyenne de 20μ, avec un minimum de 12 à 15μ et un maximum de 40μ quand des branches sont présentes, ce qui arrive rarement. Parfois, l'une des métules du pinceau est beaucoup plus longue que les autres et alors souvent cloisonnée (fig. 15b).

Métules et phialides (stérigmates) ont des longueurs identiques: 9μ en moyenne, 7μ au minimum et 11μ au maximum. La largeur moyenne des métules de 2,7μ, est un peu supérieure à celles des phialides: 2,5μ. Les métules, au nombre de 4 ou 5 le plus souvent, s'écartent les unes des autres, faisant entre elles des angles ouverts. Les phialides, disposées en verticilles de 4 à 6 au sommet des métules, sont acuminées; elles s'élargissent en leur centre et s'amincissent brusquement à leur extrémité pour donner le tube qui formera les conidies.

Les chaînes conidiennes ne sont pas disposées parallèlement et ne forment pas de colonnes. Elles sont nettement distinctes et entremêlées. Dans les chaînes, les conidies lisses, sont d'abord

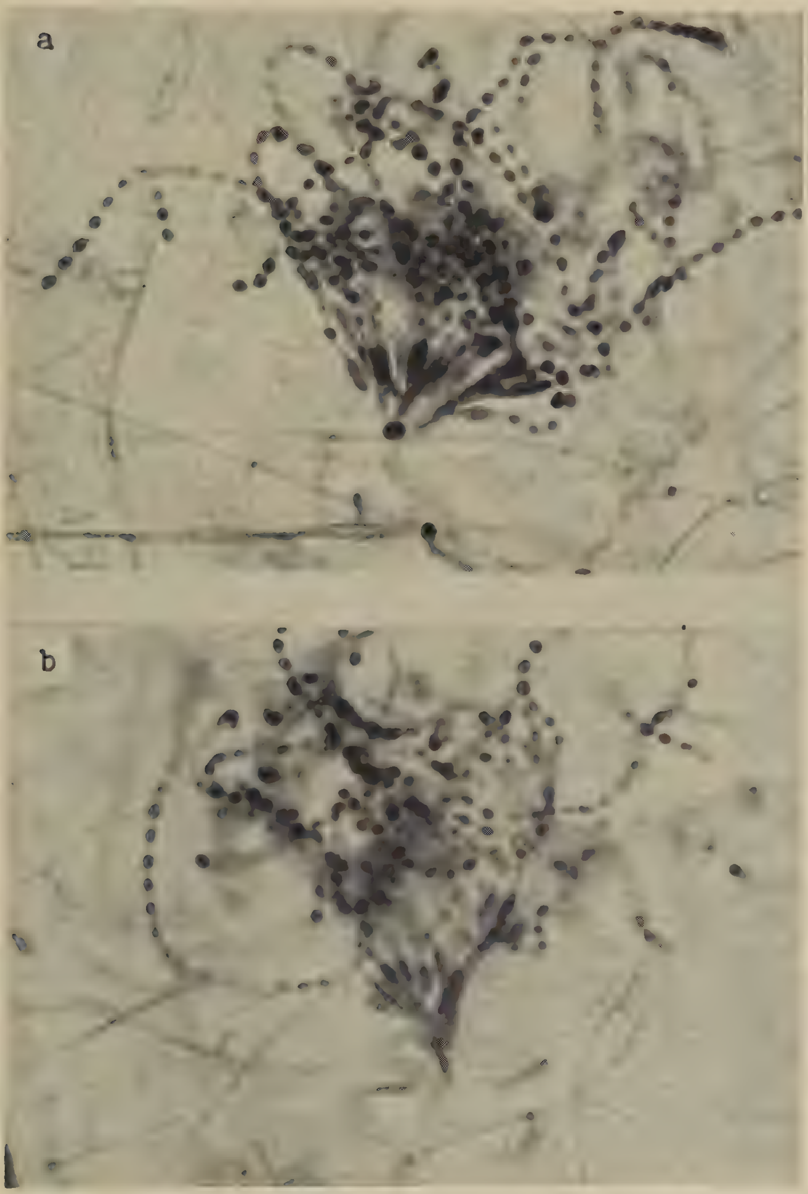


fig. 15. Pinceaux de *Penicillium marneffei* avec chaînettes de spores entremelées: b) une des métules est plus longue.
Cultures sur moût de bière; montage au bleu Coton C4B dans du lactophénol. × 670.

ovalaires, reliées entre elles par un connectif; elles sont ensuite subglobuleuses, souvent apiculées et mesurent entre 2 et 3μ de diamètre et en moyenne $2,3\mu$. On a pu observer très rarement une ou deux conidies en bout de chaîne beaucoup plus grosses que les conidies normales: c'est le „phénomène de Corda".

2°) *Sabouraud glucosé ou maltosé* (fig. 14b).

L'évolution et la constitution de la culture sont très semblables sur milieu de Sabouraud glucosé ou maltosé. Mais, à 22°, la colonie est d'emblée duveteuse et blanche, et à cette température aussi bien qu'à 27—30°, le duvet est nettement plus épais que sur moût de bière; il se teinte légèrement de vert gris au centre de la colonie et parfois entièrement en rose (rose fleur de pêcher n° 1). La formation de mèches en forme de virgule toutes orientées dans le même sens, a également été observée à 30°, 6 jours après l'ensemencement du milieu. La morphologie microscopique du champignon est la même que celle décrite précédemment.

3°) *Milieux divers*

Sur gélose peptone sans sucre à 27°, la culture est duveteuse, un peu verdâtre, à bord irrégulier; le revers et le milieu restent incolores. Le milieu aux flocons d'avoine et le milieu pomme de terre-carotte de Langeron, ne permettent à 27° qu'un faible développement du champignon en surface, sous forme d'un très léger duvet poudreux, verdâtre ou jaune verdâtre pâle. Après 15 jours, le revers de la colonie devient rose au centre ou par place, et un pigment verdâtre diffuse dans le milieu P.C. La gélatine est liquéfiée en surface après 2 mois à 22°.

4°) *Czapek*

Sur le milieu de Czapek, le champignon ne se développe que très pauvrement en surface à 22°; quelques filaments épars et blancs apparaissent 13 jours après l'ensemencement alors que le milieu se teinte de jaune verdâtre. Ce n'est que petit à petit que la sporulation apparait sous l'aspect d'une teinte rose brunâtre. A 30° aucune culture en surface n'a été observée. En remplaçant dans la formule de Czapek le saccharose par le glucose, le champignons se développe un peu mieux en surface; à 22° comme à 30°, il se forme une colonie le plus souvent glabre et mate, avec parfois un léger duvet blanc. En ajoutant au Czapek normal de l'hydrolysat de caséine, le duvet apparait plus rapidement et il reste le plus souvent blanc ou légèrement rose brun. Nous avons vérifié que les fructifications sur milieu de Czapek avaient les mêmes caractères que sur moût de bière, mais elles sont en général beaucoup moins fournies.

5°) *Cultures à* 37°

Les cultures de ce champignon à la température de 37° sont très particulières. Elles ressemblent en effet à certaines cultures bactériennes (*Bacillus subtilis*) ou à des cultures de *Geotrichum*: elles ont un aspect membraneux et glabre, soit mat, soit brillant et lisse. Elles sont sillonnées de plis radiaires en début de culture et leur couleur est blanc brunâtre. A partir du 13 ème jour de culture, les colonies peuvent se plisser de façon cérébriforme, soit en leur centre soit en quelques endroits de leur périphérie. La consistance de la colonie, membraneuse à la périphérie, peut devenir crémeuse au centre. Quand on observe un tel prélèvement crémeux au microscope, on constate qu'il est formé d'arthrospores séparées les unes des autres, qui peuvent avoir gardé la forme de bâtonnet court à bouts rectangulaires; mais dans une culture assez âgée, les bouts sont arrondis et la forme est presque sphérique, très semblable à la forme parasitaire du champignon (fig. 10c).

C) Caractères physiologiques de Penicillium marneffei.

1°) *Conditions d'obtention in vitro de la phase parasitaire du champignon.*

Il était intéressant de chercher à déterminer les conditions physiologiques qui favorisent la formation des arthrospores. Pour cela, il est préférable de cultiver le champignon en milieu liquide agité mécaniquement dans un agitateur placé à la température de 35—36°. Les milieux utilisés ont été répartis dans des fioles de Fernbach à raison de 200 à 250 ml par fiole. Après stérilisation, les fioles ont été ensemencées avec 1 ml d'une suspension d'arthrospores du champignon obtenues par culture de *P. marneffei* pendant 10 jours sur moût de bière gélosé à 37°. Dans ces conditions, le moût de bière liquide favorise la formation d'arthrospores après un certain développement du champignon sous forme filamenteuse.

Le milieu synthétique utilisé contient, par litre, des sels minéraux: PO_4H_2K: 0,36 g; $PO_4H\ Na_2$: 1,42 g; $SO_4\ Mg7H_2O$: 0,60 g; KCl: 1 g; 10 gouttes de solution olygodynamique de Berthelot. Son pH est de 6,5. Il contient en outre comme source azotée soit de l'hydrolysat de caséine 10 g/litre, soit de l'asparagine 1 g/litre et comme source carbonée pour chacune des sources azotées précédentes, soit du glucose 10 g/litre, soit du maltose 20 g/litre.

3 jours après l'ensemencement, l'examen microscopique de prélèvements entre lame et lamelle permet d'observer que le maltose semble favoriser le cloisonnement des filaments délimitant des articles courts. Sur hydrolysat-maltose, les filaments sont très courts comprenant 5 ou 6 articles et rarement ramifiés. Sur glucose, les filaments sont nettement plus longs, ramifiés et enchevêtrés, pouvant même former des pelotons. 5 jours après l'ensemencement, sur hydrolysat-maltose, on ne trouve plus que des arthrospores cylindriques, ovalaires, rondes ou piriformes. Sur hydrolysat-glucose, des éléments filamenteux à ramifications courtes et à cloisons très

rapprochées sont mêlés à des arthrospores. L'asparagine produit des filaments cloisonnés pouvant porter une spore à leur extrêmité et mêlés à des pelotons mycéliens. Dix jours plus tard, l'hydrolysat de caséine-maltose est le seul milieu à donner des formes arthrosporées mêlées à de courts filaments quatre fois plus longs que larges et septés. Des filaments longs, fins et enchevêtrés sont produits sur les milieux à l'asparagine.

En conclusion, la présence de maltose et d'une source azotée riche en acides aminés (hydrolysat de caséine) favorise la désarticulation des filaments mycéliens en arthrospores.

2°) *Etude de l'utilisation des sources carbonées*

F. Mariat a recherché en milieux gélosés, l'utilisation par le champignon de divers composés carbonés en présence d'hydrolysat de caséine ou de phosphate d'ammonium, soit à 30°, soit à 37°. L' hydrolysat de caséine seul, sans autre source carbonée, permet déjà un certain développement du champignon. Le tartrate de sodium, le citrate de sodium, le 1-arabinose, le lactose et la glycérine, donnent une croissance du champignon semblable à celle de l'hydrolysat et ne sont donc pas utilisées par le champignon: l acétate de sodium semble nettement inhibiteur; le galactose n'est que légèrement utilisé. Sur tous ces composés carbonés, le champignon ne produit pas de pigment diffusible dans le milieu. Le xylose, le levulose, le maltose et le mannitol sont bien utilisés; le saccharose légèrement moins bien; le glucose et l'amidon donnent la meilleure croissance. Dans tous ces cas où il y a utilisation de la source carbonée, un pigment soluble rouge, parfois mêlé de jaune, diffuse dans le milieu à la température de 30°; à 37° le milieu n'est pas coloré. Si l'aspect des cultures est différent, glabre à 37° et plus ou moins duvereux à 30°, on ne note aucune différence d'utilisation des composés carbonés à ces deux températures.

En présence de phosphate d'ammonium comme source d'azote, *Penicillium marneffei* se développe très mal, seulement dans la profondeur de la gélose, même en présence des sources carbonées les mieux utilisées.

3°) *Remarques sur l'utilisation des sources azotées*

Un essai systématique de l'utilisation des sources azotées n'a pas été fait, mais, suivant les différents milieux de culture employés, on peut déduire que: 1) sur Czapek, le champignon se développe mal et principalement dans la gélose du milieu, le nitrate de sodium, source azotée de ce milieu, est mal utilisé, 2) il en est de même du phosphate d'ammonium dans l'expérience précédente; 3) l'hydrolysat de caséine et la peptone sont bien utilisés, même comme seule source de C et d'N; 4) enfin, l'asparagine semble assez bien utilisée également. Il semble donc que *P. marneffei* ait besoin pour sa croissance, d'une source d'azote organique.

D) Discussion.

Pour classer *Penicillium marneffei* parmi les nombreuses espèces de *Penicillium* déjà décrites, nous nous sommes reporté aux travaux de Biourge (3) et au „Manual of the Penicillia" de Raper & Thom (15).

D'après Biourge, *Penicillium marneffei* doit être classé dans la section (ou sous-genre) *biverticillium* Dierckx parmi les non-corémigènes. En nous reportant à l'excellent manuel de Raper & Thom et après un premier examen de la forme des pinceaux, nous avons pensé classer ce champignon dans le groupe des *Biverticillata symmetrica* dans la série du *Penicillium purpurogenum* Stoll; mais en examinant plus attentivement le pinceau à fort grossissement, sa dissymétrie apparaît, certaines métules prenant naissance un peu en dessous de l'extrêmité du conidiophore. L' écartement des métules et la forme des phialides rejettent *Penicillium marneffei* de ce groupe. La forme de son pinceau le classe alors normalement dans le groupe *Asymmetrica divaricata* et dans le série du *Penicillium janthinellum*. Il a en effet plusieurs caractères communs avec *Penicillium janthinellum* Biourge: une croissance rapide sur moût de bière gélosé, une texture laineuse avec conidiophores prenant naissance sur les filaments aériens entremêlés, des phialides qui s'amincissent brusquement en un tube formant les conidies, enfin des chaînes conidiennes entremêlées.

Bien que les conidiophores ne soient pas dressés, mais mélangés aux filaments aériens, nous pensons que les caractères suivants l'apparentent également àlasérie du *Penicillium citrinum*: la croissance très restreinte sur milieu de Czapek, les cultures plissées radiairement, le conidiophore court, le nombre assez élevé des métules et des phialides et la forme des conidies. Dans cette série, *Penicillium marneffei* serait à rapprocher de *Penicillium citrinum* Thom. Ce champignon est un saprophyte des régions tropicales et subtropicales et en particulier du sud-ouest de l'Océan Pacifique. Il a été isolé à plusieurs reprises de produits pathologiques sans caractère de pathogénicité très net. D'après Bailey & Cavallito (2), l'azote nitrique et ammoniacal serait mieux utilisé par ce champignon que l'azote organique, ce qui est tout à fait contraire aux besoins azotés de *P. marneffei*. Mais, Raper & Thom, signalent l'existence de mutants poussant très pauvrement sur Czapek et n'utilisant pas l'azote nitrique. *P. marneffei* se distingue cependant de *P. citrinum* par une très bonne croissance sur moût de bière gélosé, par un pinceau nettement plus ouvert, par des métules beaucoup plus courtes (7 à 11 μ au lieu de 12 à 20 μ) et par des chaînes conidiennes nettement indépendantes et entremêlées n'ayant aucune tendance à se rassembler en colonnes. Le pigment soluble diffusible dans la gélose est nettement rouge, parfois orangé rouge et rarement jaune; aucune gouttelette d'exsudat n'apparait sur la colonie.

Enfin, *Penicillium citrinum* diffuse dans le milieu un antibiotique antibactérien: la citrinine; or aucune action antibiotique de *P. marneffei* n'a pu être mise en évidence sur le staphylocoque Londres, *Bacillus subtilis*, et deux souches de *Bacillus cereus* en présence ou en absence de pénicillinase. Pour mettre en évidence cette action on a utilisé la méthode des stries perpendiculaires en boite de Pétri sur gélose de Sabouraud glucosée et sur gélose au bouillon de viande glucosée. Les stries bactériennes ont été faites après 8 jours de développement du champignon à 27 et à 37°.

Nous considérons donc *P. marneffei* comme une espèce nouvelle. A notre avis, son rôle pathogène est essentiel et nous proposons donc que les noms des auteurs qui l'ont découvert soient joints à celui qui en a fait la description botanique. Le champignon devient alors: *Penicillium marneffei*, SEGRETAIN, CAPPONI, SUREAU.

RÉSUMÉ

1) Une espèce nouvelle de *Penicillium, P. marneffei*, SEGRETAIN, CAPPONI, SUREAU, a été isolée d'un rongeur du Centre Viet Nam, *Rhizomys sinensis* ou rat de bambou, provoquant la mortalité de cet animal en captivité,

2) Ce champignon est expérimentalement pathogène pour la souris le hamster et le rat, atteints d'une réticulose macrophagique semblable à l'histoplasmose ou à la leishmaniose.

3) Le champignon se développe dans les cellules réticulaires où il se présente sous forme ronde, ovale ou légèrement allongée. Il se multiplie par cloisonnement transversal.

4) En culture à 37°, *P. marneffei* donne des cultures glabres, lisses ou plissées, de consistance crémeuse, ressemblant à une culture bactérienne et formées d'arthrospores.

5) Par ses caractères botaniques, le champignon doit être considéré comme une espèce nouvelle, à rapprocher de *P. janthinellum* et de *P.citrinum*.

SUMMARY

1) A new species of *Penicillium, P. marneffei* SEGRETAIN, CAPPONI, SUREAU, has been isolated from a rodent of the center of Viet Nam, *Rhizomys sinensis* or bamboo rat; it causes the death of this animal living in captivity.

2) This fungus is experimentally pathogen for mice, hamsters and rats: it produces a reticulosis similar to histoplasmosis or leishmaniosis.

3) The fungus grows in the reticulo-endothelial cells in which its form is round, oval or slightly elongated. It multiplies by scissiparity.

4) At 37° C the colony of *P. marneffei* is glabrous, smooth or folded, resembling that of Bacteria and formed by arthrospores.

5) By its botanical caracters, the fungus is considered as a new species to be classed near *P. janthinellum* and *P. citrinum*.

BIBLIOGRAPHIE

1. Aimé, P., Creuzé, P. & Kresser, H., Mycose pulmonaire à *Penicillium crustaceum* avec signes cliniques et aspect radiologique d'abcès du poumon. — Presse Méd., 1933, **41,** 761—763.
2. Bailey, J. H. & Cavallito, C. J., The utilisation of various carbon and nitrogen sources by *Penicillium citrinum* Thom. — J. Bact., 1944, **47,** 411.
3. Biourge, P., Les moisissures du groupe *Penicillium* Link. Etude monographique — La Cellule, 1923, **33,** 5—332.
4. Braunstein, L., Fréquence des Mycoses pures ou associées à la tuberculose — Thèse de Médecine, Paris 1927.
5. Capponi, M., Sureau, P. & Segretain, G., Penicillose de *Rhizomys sinensis* — Bull. Soc. Pathol. exot., 1956, **49,** 418—421.
6. Coutelen, F., Biguet, J., Cochet, G., Mullet, S. & Doby-Dubois, M., Etude d'un champignon nouveau isolé d'une tumeur mycosique pulmonaire — Ann. Parasit. hum. comp., 1955, **XXX** n° 4, 395—411.
7. Delore, P., Coudert, J. & Lambert, R., Penicillose bronchique avec manifestations septicémiques — Presse Méd., 1957, **65,** 1916.
8. Delore, P., Coudert, J., Lambert, R. & Fayolle, J., Un cas de mycose bronchique avec localisations musculaires septicémiques. Press Méd., 1955, **63,** 1580—1582.
9. Drouhet, E. & Schwarz, J., Comparative studies with 18 strains of *Histoplasma*. Morphology in tissues and virulence of African and American strains.— J. Lab. Clin. Med., 1956, **47,** 128—139.
10. Drouhet, E. & Segretain, G., Histoplasmose expérimentale chez le hamster doré — Ann. Inst. Pasteur, 1952, **83,** 381.
11. Gilliam, J. S. & Vest, S. A., Penicillium infection of the urinary tract. — J. Urology, 1951, **65,** 484—489.
12. Mantelli, C. & Negri, G., Ricerche speiimentali sull'agente eziologico di micetoma a grani neri (*Penicillium mycetogenum* n.f.) — Giorn. Acad. Med. Torino, 1915, **78,** 161.
13. Mariat, F. & Drouhet, E., Sporotrichose expérimentale du hamster. Observation de formes asteroïdes de *Sporotrichum*. — Ann. Inst. Pasteur, 1954, **86,** 485.
14. Oberthür, R. & Dautenay, H., Repertoire de couleurs — Publié par la Soc. franç. des Chrysanthémistes, 1905.
15. Raper, K. B. & Thom, C., A manual of the *Penicillia* — Baltimore 1949, Williams et Wilkins C° Edit.
16. Segretain, G. & Drouhet, E., Blastomycoses expérimentales du hamster doré — Ann. Inst. Pasteur, 1955, **89,** 593.
17. Talice & Mackinnon, J. E., *Penicillium bertai* n. sp., agent d'une mycose broncho-pulmonaire de l'homme. — Ann. Parasit., 1929, **7,** 97.

ON BLACK AND WHITE PIEDRA

by

K. B. BOEDIJN
'*s-Gravenhage*

(28.V.1959)

INTRODUCTION

Of the rather restricted number of fungi parasitizing men, most species belong to the *Deuteromycetes* and only a few can be placed in the *Ascomycetes.*

Whereas nearly all species are attacking the skin, some grow directly upon the hairs, forming there small hard bodies. The affection is known as piedra, a South American name now generally used to indicate this mild disease.

We may distinguish between black piedra, where the bodies are dark colored, nearly black, and white piedra where they are of a pale color. Black piedra is especially noteworthy, because here asci are formed within the nodules.

BLACK PIEDRA

The first species of black piedra was described as a *Trichosporon* but later the genus *Piedraia* was created by DA FONSECA & AREA LEAO (9) to accomodate these peculiar fungi, which have nothing in common with *Trichosporon.*

Up till now 7 species of *Piedraia* are described, viz. *Piedraia Hortai* (BRUMPT) DA FONSECA & AREA LEAO Inst. Oswaldo Cruz, Suppl. das Mem. 4. 124—127. 1928 *type species.*

Piedraia paraguayensis DELAMARE & GATTI Bull. Acad. Med. III 99. 500—503. 1928.

Piedraia Sarmentoi PEREIRA f. Rev. Med. Cirurg. Brasil 38 49—52, 1929.

Piedraia venezuelensis BRUMPT & LANGERON Ann. Parasit. hum. Comp. 12, 155—158, 1934.

Piedraia surinamensis DODGE Medical Mycology p. 133, 1935.

Piedraia colombiana DODGE Medical Mycology p. 133, 1935.

Piedraia javanica BOEDIJN & VERBUNT Mycopathologia 1, 196, 1938.

The disease, which for a long time was only known to occur in South America, was later also discovered in South Asia, namely in Tonkin, Annam and Indonesia. As the Indonesian material was different from the South American species it has been described as a new species under the name of *Piedraia javanica.*

Afterwards doubt was expressed as to the validity of the different species and it was suggested by CIFERRI, CHAVES BATISTA & SYLVIO CAMPOS (4) that there is only one species of *Piedraia,* viz. *Piedraia Hortai* and that all other names are synonyms.

To settle this question one has to compare fruiting material of the different species. I am greatly indebted to Dr CIFERRI for placing at my disposal material of *Piedraia Hortai* from Recife, Pernambuco, Brasil. *Piedraia javanica* was represented by several new collections made during the year 1948 in Djakarta. So a comparison of at least 2 species was possible. As the accompanying figure 1 shows, there cannot be the slightest doubt, that we have to deal with 2 different species. The differences being in size and shape of the ascospores. For *Piedraia javanica* the spore measurements of the original material were 20—60 × 4—12μ (without the appendages). The new material is nearly identical viz. 37—55 × 6—11μ. In the description of *Piedraia Hortai* the spores are said to be 30 × 10μ. However CIFERRI et all. (4) are giving the measurements as 13½—30½ × 1,3—2,7μ. Those data certainly are attri-

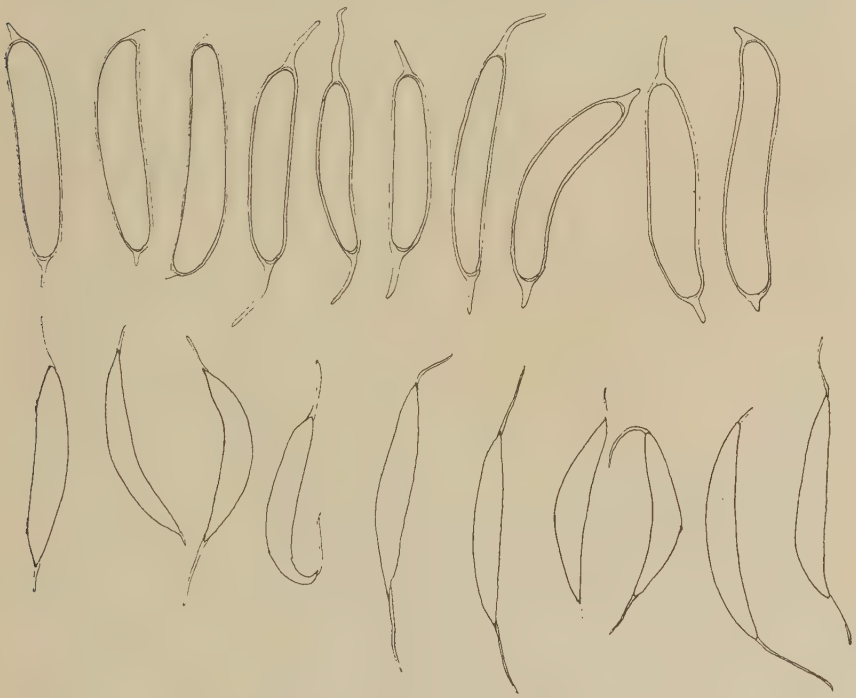


Fig. 1. Upper row Ascospores of *Piedraia javanica.*
Bottom row Ascospores of *Piedraia Hortai.*

butable to an error as I find the ascospores of the material from Recife as 34—47 × 5½—7μ. Anyhow it is evident, that the ascospores of *Piedraia javanica* are larger and especially broader than those of *Piedraia Hortai*. But the most striking differences are found in the spore shape. *Piedraia javanica* has thickwalled spores with broadly rounded tips, while those of *Piedraia Hortai* are thinwalled with the tips strongly acuminated. Also the appendages in *Piedraia javanica* are coarser and often much shorter than in *Piedraia Hortai*. In our original description of *Piedraia javanica* we thought that the exosporium was loosely surrounding the spore body. This however is not the case; only at the tops the exosporium is often free from the endosporium.

As I could prove, that *Piedraia javanica* and *Piedraia Hortai* are 2 different species, so the other representatives of the genus *Piedraia* may as well be good species too. Only a renewed study of sporulating material can solve the question. In the light of our investigations it appears premature to assemble all species under the name *Piedraia Hortai*.

Our genus *Piedraia* was first considered to belong to the *Moniliales*. DODGE (8) placed it in the *Endomycetales* and LANGERON (13) put it in the *Microthyriales*. Last named author thinks that our fungi are belonging to the "Astérinées" of ARNAUD, a biological group consisting of leaf parasites with mostly well developed mycelium and typical flat, shield shaped fructifications, occuring chiefly in wet tropical countries. Our *Piedraia* species however are wholly different and the theory that they can only grow in the climate of the "Astérinées" is misleading. As so many tropical plants they prefer a hot, humid environment.

The morphology of the ascocarp of *Piedraia* led CIFERRI et all. (4) rightly to the conclusion, that the genus belongs in the *Myriangiales* where it comes near the family *Myriangiaceae*. We find the same cushion shaped ascocarps, with the asci irregular distributed in locules throughout the stroma. Mostly each locule contains 1 ascus, but in *Piedraia javanica* sometimes more than 1 ascus is found in a locule. But in the shape of the ascospores *Piedraia* differs from the representatives of the *Miriangiaceae*. So in the order *Myriangiales* it cannot be accomodated into any of the families and a separate family the *Piedraiaceae* had to be created.

Now we know, that the *Myriangiales* belong to the subclass *Loculoascomycetes* and that those fungi are characterised among other things by the presence of bitunicate asci. But in *Piedraia* the ascus is thinwalled and certainly not bitunicate. Notwithstanding this deviation of the rules, our genus cannot be placed elsewhere and we have to accept the fact, that there are also *Loculoascomycetes* without bitunicate asci.

WHITE PIEDRA

A piedra of the hairs, which is much rarer than the black piedra, at least in Indonesia, is the socalled white piedra. This affection was formerly attributed to *Trichosporon Beigeli* RABENH. LODDER & KREGER VAN RIJ (15) however consider this to be a nomen dubium as the original description is too vague to be of any use. According to these authors the real cause of white piedra is *Trichosporon cutaneum* (DE BEURMAN, GOUGEROT & VAUCHER) OTA. In Bogor one case was detected and the fungus in question taken in pure culture.

Trichosporon cutaneum (DE BEURMAN, GOUGEROT & VAUCHER) OTA Ann. Parasit. hum. Comp. 4. 2—13. 1926 (Fig. 2)

Colonies after about 3 month 4 cm in diam. waxy; in the centre slightly elevated and here coarsely plicate and bullate. Border flat, smooth, weakly powdery under a hand lense. Color dirty ocher, about claycolor Ridgway, but decidedly more grey.

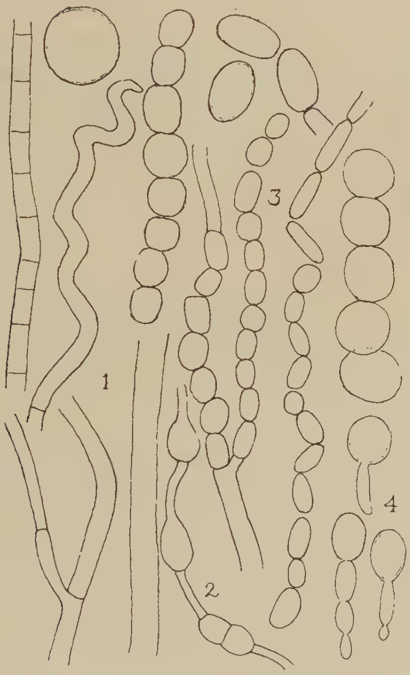


Fig. 2. *Trichosporon cutaneum.*
1 mycelial threads, 2 Racquet mycelium, 3 threads falling apart into arthrospores. 4 Budding arthrospores.

Mycelium strongly developed, sparingly branched, the separate threads $1^1/_2$—5 μ in diam. At first without crosswalls and containing much oil, afterwards richly septated. There are always a small number of tortuous and spirally coiled hyphae and threads with swollen segments. By continual septation, constriction and swelling of the separate cells, the threads are transformed into chains of arthrospores. Arthrospores very variable as to shape and dimensions. Those originating from thick hyphae are of course the largest. They are globose, elliptic till elongated, sometimes attenuated near one of the poles and with a slightly thickened cellwall; 3—$12^1/_2$ μ in diam. or 3—13 × $3^1/_2$—6 μ. Most frequent are elliptic spores $6^1/_2$—$9^1/_2$ × 4—6 μ, with the wall about $^1/_2$ μ thick. Arthrospores budding at one of the poles with one or a short row of ellipsoid cells.

Literature

1. AARS, C. G. (1930) Piedra. Arch. Derm. Syphil. **22,** 401—409, 9 fig.
2. BOEDIJN, K. B. & VERBUNT, J. A. (1938) Annotations about dermatomycoses in Batavia. Mycopathologia **1,** 185—198, 4 fig., 6 pl.
3. BRUMPT, E. & LANGERON, M. (1934) Considérations sur la piedra de l'Amérique du sud à l'occasion d'un cas provenant du Vénézuéla, description d'une espèce nouvelle, *Piedraia venezuelensis* n. sp. Ann. Parasitol. hum. Comp. **12,** 134—161, 32 fig. 1 pl.
4. CIFERRI, R., BATISTA, A. CH. & SYLVIO CAMPOS. Taxonomy of *Piedraia Hortai* and systematic position of the *Piedraiaceae* family. University of Recife, Brazil Institute of Mycology, Publication no. 45, 1956, 9 p. 8 fig.
5. CIFERRI, R., BATISTA, A. CH., CIFERRI, F. & I. DA COSTA ALECRIM. The culture of *Piedraia Hortai* in natural media. University of Recife, Brazil, Institute of Mycology Publication no. 51, 1956, 9 p.
6. DANG VAN-NGU (1939) La Piedra noire au Tonkin et en Annam. Ann. Parasitol **17,** 359—363, 1 pl.
7. DELAMARE, G. & GATTI, C. (1928) Sur la piedra du Paraguay. Bull. Acad. Med. **III,** 99, 500—503.
8. DODGE, C. W. (1935) Medical Mycology. 131—134, fig. 15.
9. DA FONSECA, O. O. R. (1930) O genero *Trichosporon,* trichosporeses ou trichomycoses nodosas, piedra europea, piedra asiatica e piedra americana. Rev. Med. Cirurg. Brasil **38,** 251—261, 5 fig.
10. DA FONSECA, O. O. R. & DE AREA LEAO, A. E. (1928) Sobre os cogumelos da piedra brasileira. Mem. Inst. Oswaldo Cruz, Suppl. **4,** 124—127, 2 pl.
11. HORTA, P. (1911) Sobre uma nova forma de piedra. Mem. Inst. Oswaldo Cruz **3,** 86—107, 2 pl.
12. LAMPE, P. H. J. (1940) Over piedra en het voorkomen van piedra te Batavia. Geneesk. Tijdschr. voor Nederlandsch Indie, **80,** 1519—1525, 12 fig.
13. LANGERON, M. (1929) Les astérinées parasites de l'homme. La Piedra. Ann. Parasitol **7,** 309—324.
14. LANGERON, M. (1945) Précis de Mycologie, 23—25, 81, 544—547, 649.
15. LODDER, J. & KREGER-VAN RIJ, N. J. W. (1952) The yeasts a taxonomic study, XI and 713 pp., 268 fig.
16. PENA CHAVARRIA, A. & WERNER ROTTER, (1937) Die Kolumbianische Piedra. Arch. Schiffs-u. Tropenhyg. **37,** 73—87, 16 fig.
17. PEREIRA FILHO (1930) Culturas da piedra brasileira. *Piedraia Sarmentoi* n. sp. Rev. Med. Cirurg. Brasil **38,** 49—50, 6 pl.
18. VIEGAS, A. P. (1943) *Piedraia Hortai* (BRUMPT) FONSECA & AREA LEAO. Bragantia **3,** 37—40.

SYNOPSIS OF A NEW CLASSIFICATION OF THE ECTOTROPHIC MYCORRHIZAE ESTABLISHED ON MORPHOLOGICAL AND ANATOMICAL CHARACTERISTICS.

Tadeusz Dominik

Forest Microbiological Laboratory,
Forest Research Institut Szczecin, Poland

(12.III.1959)

In the course of about 30 years the author examined mycorrhizal infections in natural plant associations. He proposes a new classification of the ectotrophical mycorrhizae.

The original division of mycorrhizae (by Frank) into two principal types: ectotrophic and endotrophic mycorrhizae, will be retained.

Burgeff's classification of the endotrophic mycorrhiza seems to be incontestable.

However, the classification of ectotrophic mycorrhiza is insufficient and needs changes and corrections.

Therefore the author proposes a new classification of ectotrophic mycorrhiza:

1. Fungus mantle (hyphal sheath) simple, root simple or ramifying 2
– Mantle with two layers. Mantle enveloping single or ramifying roots or two and more rootlets which grow together and have simple mantles and form tubers of different sizes. The tubers are enveloping in own independent mantles 8
2. Mantle felt-like (prosenchyma) 3
– Mantle pseudoparenchymatous (synenchyma) 6
3. Mantle loosely woven, enveloping the short-root, like a felt, with gaps in it. There are often strands of mycelium. These strands are not densely woven forming no real rhizomorphs: *subtype A*
– Mantle densely and regularly woven, without any gaps, sometimes appearing to be pseudoparenchymatous 4
4. Surface of the fungus mantles smooth or only covered with very short and mild nap of hyphae: *subtype B*
– Surface of the mantle covered with hyphae of different struc-

ture: bristles (setae), sterile tubes (cystids), rhizomorphs, very large nap of hyphae 5

5. Mantle covered with numerous rhizomorphs which under the binocular looks like a veil or with large nap of hyphae:

subtype C

– Mantle covered with bristles. These are short or long, straight, bent or curved, the ends are pointed, the bases are swollen or not swollen:

suptype D

– Mantle covered with many sterile tubes (cystids) in a palisade arrangement:

subtype E

6. Surface of the mantle smooth or only covered with single and isolated hyphae:

subtype F

– Surface of the mantle covered with numerous hyphae which under the binocular look like root hairs or with bristles or rhisomorphs 7

7. The hyphae which grow out of the mantle are stiff and their ends are never pointed; generally the whole mantle is almost black or dark brown-black:

subtype G

– The hyphae which grow out of the mantle are soft. They often form loose hyphal strands or there is another layer of loose hyphal strands around the pseudoparenchymatous layer:

subtype H

– On the surface of the pseudoparenchymatous mantle there are bristles of different quantity:

subtype I

8. Mycorrhizae are present only as simple fungus roots . . . 9

– Mycorrhizae (two or many) are grown together in a compound, tuberlike. Every short root has its own fungus mantle and the whole tuber has a common mantle. The two kinds of mantles have the same structure:

subtype J

9. The outer layer of the mantle is felt-like 10

– The outer layer of the mantle is pseudoparenchymatous . . 11

10. The outer layer of the mantle is dark brown. The inner layer has a different colour and a different structure. One fungus is superimposed on the other:

subtype K

– The outer layer and the inner layer of the mantle have the same colour but a different structure. Both layers are formed by the same fungus:

subtype H

11. The surface of the outer layer is composed of elliptical brown or black-brown cells. The inner layer is colourless, felt-like and densely woven. The structure of the mantle looks like that of the „Perithecia":

subtype L

– The outer layer of the mantle is dark-brown. The inner layer has different colour and different structure. One fungus is superimposed on the other:

subtype K

CLASSIFICATION OF THE MYCORRHIZAL SUBTYPES

Subtype A

1.	Fungus mantle colourless (under the binocular white-grey)	genus Aa
2.	Fungus mantle (fresh) under the binocular cream-coloured	genus Ab
3.	Fungus mantle under microscope yellow or yellowish	genus Ac
4.	Fungus mantle under the binocular vivid lemon coloured	genus Ad
5.	Fungus mantle under the microscope light ochre	genus Ae
6.	Fungus mantle under the microscope brownish to brown	genus Af
7.	Fungus mantle dark-brown to greenish-black	genus Ag
8.	Fungus mantle black-blue (ink-blue)	genus Ah

Subtype B

1.	Fungus mantle colourless (under the binocular white-grey)	genus Ba
2.	Fungus mantle under the binocular cream-coloured	genus Bb
3.	Fungus mantle under the microscope yellow to yellowish	genus Bc
4.	Fungus mantle under the microscope light ochre	genus Bd
5.	Fungus mantle under the binocular rose to pink (this colour is disappearing into alcohol)	genus Be
6.	Fungus mantle under the microscope vivid brown	genus Bf
7.	Fungus mantle under the microscope dark-brown to black-brown	genus Bg

Subtype C

1.	Fungus mantle colourless or clear white	genus Ca
2.	Fungus mantle under the binocular cream-coloured	genus Cb
3.	Fungus mantle under the microscope yellow to yellowish	genus Cc

4. Fungus mantle under the microscope light ochre . . genus Cd
5. Fungus mantle under the binocular pink to carmine genus Ce
6. Fungus mantle light brown to dark-brown. . . . genus Cf
7. Fungus mantle dark grey (under the microscope ligth greenish) . . . . genus Cg

Subtype D

1. The mantle is covered with short bristles, the length of which is never longer than ten times the width of the base. The colour of the mycelium is under the binocular greyish white to dark grey: the hyphae under the microskope are colourless . . . . genus Da
2. The mantle is covered with very long bristles which are straight or not straight. The bristles are very much longer than the width of the base (at least eleventimes). The colour is the same as number one genus Db

Subtype E

1. Colour of the mantle under the microscope whitish-grey or yellowish-grey. The single hyphae are colourless or yellowish-brown. The mantle varies: one layer is colourless, the other yellow-brown . . . . genus Ea

Subtype F

1. Fungus mantle colourless (under the binocular grey-whitish) . . . . genus Fa
2. Fungus mantle under the binocular cream-coloured genus Fb
3. Fungus mantle under the microscope yellow to golden-yellow . . . . genus Fc
4. Fungus mantle under the microscope ligth ochre genus Fd
5. Fungus mantle (fresh) under the binocular pink to red . . . . genus Fe
6. Fungus mantle brown . . . . genus Ff
7. Fungus mantle dark brown (bronze) . . . . . . genus Fg
8. Fungus mantle black . . . . genus Fh
9. Fungus mantle olive-grey . . . . genus Fi

Subtype G

1. Colour of the mycelium is black, seldom brown-black. The surface of the mantle is covered with hyphae which are straight and smooth, projecting at right angle from the surface. Sometimes the mantle is covered with hyphal strands. . . . . . genus Ga
2. Colour of the mycelium is bronze to light brown, the surface of the mantle is smooth or covered with yellowish hyphae, projecting at right angle from the surface, not straight . . . . genus Gb

Subtype H

1. Fungus mantle colourless or under the binocular grey-whitish . . . . genus Ha

2. Fungus mantle under the binocular cream-coloured genus Hb
3. Fungus mantle under the microscope yellowish to yellow . . . . genus Hc
4. Fungus mantle under the microscope light ochre . . genus Hd
5. Fungus mantle (fresh) under the binocular pink to vivid red. . . . . genus He
6. Fungus mantle brown . . . . genus Hf
7. Fungus mantle dark bronze . . . . genus Hg
8. Fungus mantle light citron-coloured . . . . . . genus Hh

Subtype I

1. Bristles colourless
   - a. Bristles pointed, stiff. Fungus mantle yellowish-brown. genus Ia
   - b. Bristles never pointed, flabby. Fungus mantle white or colourless . . . . genus Ic
2. Bristles brownish to brown
   - a. Bristles with thick walls, feebly coloured. Fungus mantle dark brown. . . . . genus Ib
   - b. Bristles with thin walls, intensively coloured. Fungus mantle brown . . . . genus Id

Subtype J

1. Fungus mantle colourless or light yellowish-greyish, under the binocular whitish-grey to cream-coloured. Tubers of different sizes. . . . . genus Ja

Subtype K

1. The outer layer of the mantle is formed by dark bronze-coloured hyphae (burnt siena). The hyphae are neither stiff nor thick. This fungus is *Mycelium radicis atrovirens*. The inner layer is of a different structure and colour . . . . genus Ka
2. The outer layer of the mantle is formed by violet or black-bronze-coloured, very stiff and thick hyphae becoming pseudoparenchymatous. In some cases, the whole outer layer is pseudoparenchymatous. This fungus is *Cenococcum graniforme*. The inner layer is of different structure and colour . . . . genus Kb
3. All features of the mantle are like those of number two, but the hyphae are smaller than the hyphae of *Cenococcum graniforme*. There are often „nests" and pieces of pseudoparenchyma . . . . genus Kc

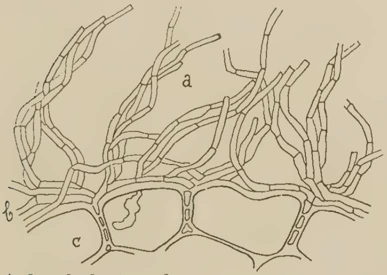
Subtype L

1. The surface of the outer layer is formed by elliptical, brown or black-brown bristles. The inner layer is colourless and felt-like and of a densely woven structure. The bristles are very stiff and sharply pointed. The structure of the mantle looks like that of the „Perithecia" . . . . genus La

Cross-Sections trhough the Fungus Mantles of Ectotrophic Mycorrhizae

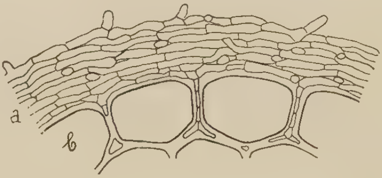
Subtype A to L. (Sectors, Schematic)

Subdtype A



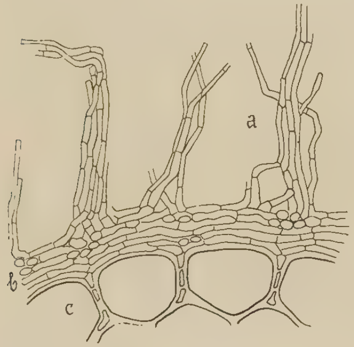
a) hyphal strands
b) a thin loosely woven mantle, sometimes absent
c) cortical cells of the root (beech)

Subtype B



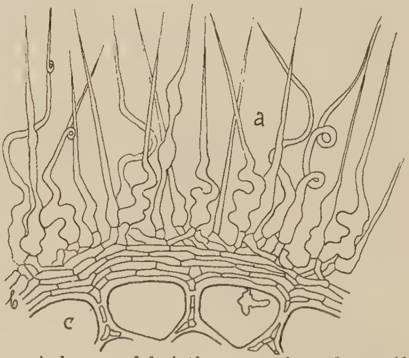
a) mantle densely woven with a smooth surface
b) cortical cells of the root (spruce)

Subtype C



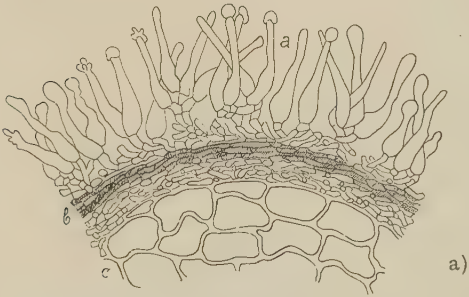
a) rhizomorphs
b) mantle denseley woven
c) cortical cells of the root (Scots pine)

Subtype D



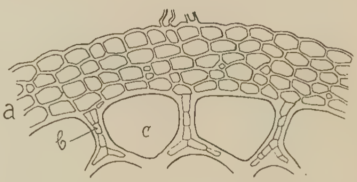
a) layer of bristles covering the cuff of the fungus mantle
b) mantle densely-woven
c) cortical cells of the root (spruce)

Subtype E



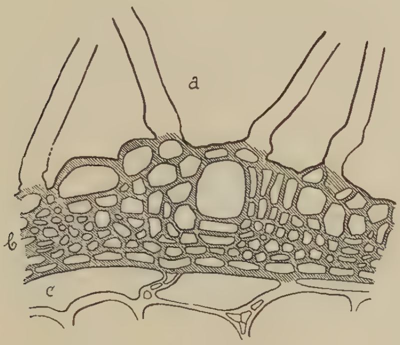
a) layer of sterile tubes (cystids)
b) denseley woven mantle, there is a distinct difference in colour and position of the hyphae
c) cortical cells of the root (fir)

Subtype F



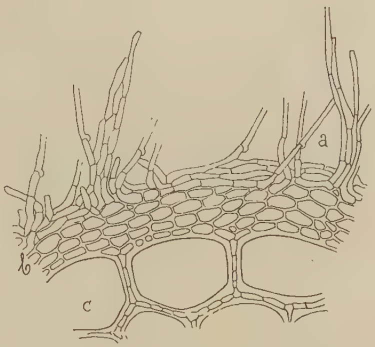
a) mantle pseudoparenchymatous wit a smooth surface
b) rich fungal development between the cells of the outer cortex (this is not always the case)
c) cortical cells of the root (Scots pine and Polish larch)

Subtype G



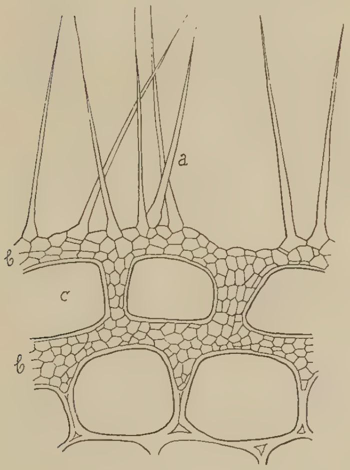
a) layer of stiff and thick hyphae (not always formed distinctly)
b) thick pseudoparenchymatous mantle with cells of different size
c) cortical cells of the root (Polish larch)

Subtype H



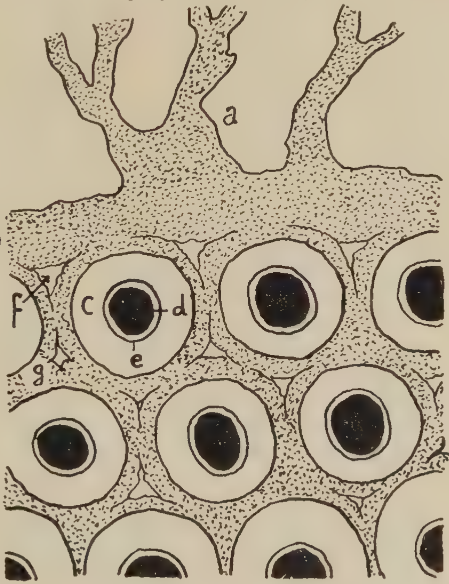
a) the surface of the mantle is feltlike with numerous single communication-hyphae and rhizomorphs extending into the surrounding soil
b) pseudoparenchymatous mantle
c) cortical cells of the root (no tree species noted)

Subtype I



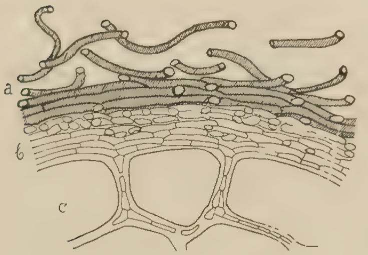
a) layer of bristless (this layer is often very irregularly formed)
b) a pseudoparenchymatous mantle. The mycelium separates the cortical cells of the root; these cells seem to be bedded in the fungal parenchyma
c) cortical cells of the root (poplar)

Subtype J Tuberous mycorrhizae



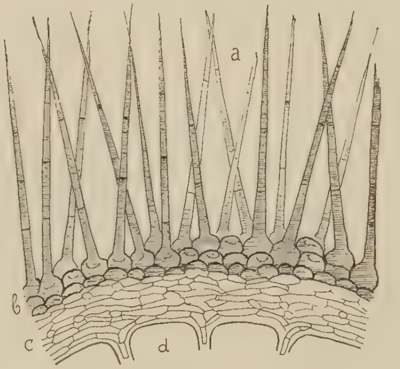
a) layer of rizomorphs
b) mantle surrounding the whole tuber
c) root cortex of one of the enclosed rootlets
d) dark central cylinder (stele) of the enclosed rootlets
e) endodermis
f) gap between the fungus mantle of the rootlets
g) single fungus mantle of one of the rootless of the tubercle (Pinus montana)

Subtype K



a) mantle formed of dark brown hypand covering the real fungus mantle
b) the real mantle
c) cortical cells of the root (Scots pine)

Subtype L



a) layer of long dark brown bristles; they are large and the ends are pointed
b) layer of elliptical cells and the bases of bristles
c) mantle of densely woven colourless hyphae
d) cortical cells of the root (Quercus pedunculata)

NOTES

Note 1. The following colours are disappearing in alcohol: white, cream, pink to carmine. It is necessary to note the unstable colours of the fresh root's material by collecting.

Note 2. The genera of subtype K should be submitted to further examination in order to find criteria which enables us to have a more exact classification.

Note 3. It was useful to perform a colour-synopsis with the names of colours for my classification of mycorrhizae. I have this synopsis in my laboratory.

Note 4. It is very important to know, that the colours of mycorrhizae and those of fungal mantles are quite different things. The colours of mycorrhizae change with the age of mycorrhizae. But the other colours are the results of the interference of the unalterable or not easily alterable colour of fungal mantle. It turns rapidly darker due to the tannin layer in the cortical paremchyma of the root. The coloured tannin layer does not exist in the very young mycorrhizae. It is slightly brown or tawny in the mycorrhizae being in the full development (mature). It is dark brown to black brown in the old mycorrhizae. Then it is necessary to examine the mycorrhizae on the microscopic sections of 15—25 μ thickness. Only those colours, which are disappearing in alcohol must be circumscribed on the fresh roots under the magnifying glass or under the binocular. In general should be investigated for the classification of the mycorrhizae only the mature stages of the mycorrhizae. The youngest and the oldest stages of the mycorrhizae can be used for the classification exclusively by the expert specialists. On the tree roots all the stages of development of the mycorrhizae exist simultaneously.

It is most likely that, in the future, one has to extend and modify the classification. The classification presented here may contribute to an easier way of comparing notes among research workers concerned with mycorrhizal problems.

Some objections may be raised against my classification(above all that the same fungus takes part in forming the mycorrhizal associations of different subtypes and genera).

In some cases there may be a reason for this reproach. However the described units are so permanent and so frequent in nature that they have to be classified in any case. Wether the mycorrhiza is formed by one fungal species or by another, is a question which will be answered by further investigations.

Dominik, T. — Projekt nowego podzialu mikoryz ektotroficznych oparty na cechach morfologiczno-anatomicznych. Roczniki Nauk Lesbych, **XIV**, 1956, pp. 223—245.

Dominik, T. — Proposal of a New Classification of the Ectotrophic Mycorrhizae Established on Morphological and Anatomical Characteristics. Mimeographed in Reinbek b. Hamburg.

LIBRI NOVI

Ainsworth, G. C. and Austwick, P. K. C.: Fungal Diseases of Animals. Review Series no. 6 of the Commonwealth Bureau of Animal Health. — Commonwealth Agricultural Bureau, Farnham Royal, Bucks, England. — Medium 8vo, xii, 148 pp. 8 colored and 17 black and white illustrations. Over 650 references. Price: 20 shillings.

This concise volume represents a more than welcome addition to the mycological literature on veterinary mycology. This is the first time that veterinary-mycological data scattered all over the world literature are critically sifted and presented in one very handy volume. Beside mycoses which are common to man and animals, like cryptococcosis, sporotrichosis, coccidioidomycosis, etc. thorough information is given about mycoses which concern mainly if not exclusively household and wild animals. like aspergillosis, bovine mycotic abortion, mycotic mastitis, etc. An important and interesting chapter deals with mold toxicoses and poisonous fungi. The chapters are uniformily organized: they give the definiton of the mycosis, the host, the geographical distribution, symptomatology, pathology, mycology, serology (if available), epidemiology and treatment, if any. The reader will find as a great help that in the group of common dermatophytes (*Trichophyton*, *Microsporum*, etc.) beside the current nomenclature, also the older terms according to Sabouraud are given. This prevents a greater confusion in the constantly changing nomenclature. The literature references are worldwide. The illustrations are excellent and well chosen. The production of the book is excellent, it is printed by litography. This book fills a long standing gap and it can be warmly recommended not only for student of veterinary medicine, but also for medical mycologists and mycologists in general.

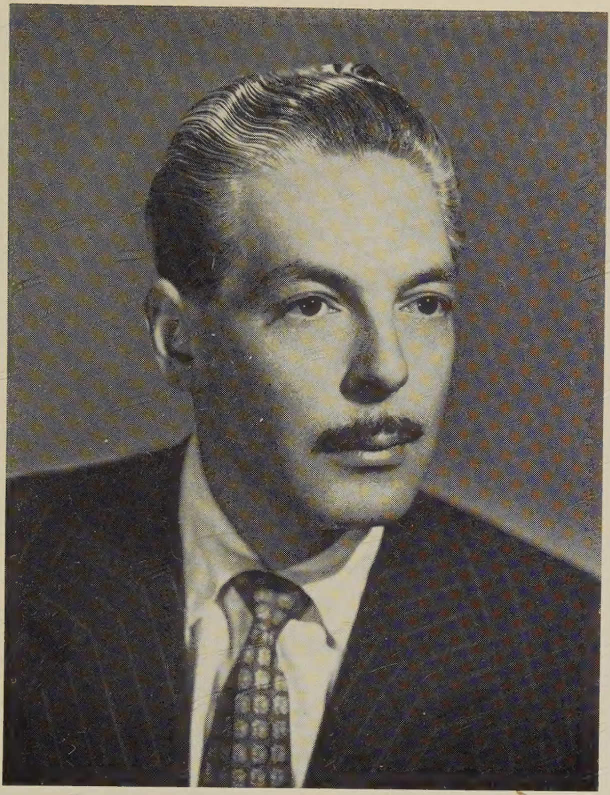
Tibor Benedek

WM. BRIDGE COOKE

Robert A. Taft Engineering Center,
Public Health Service
U.S. Department of Health, Education and Welfare
Cincinnati, Ohio, U.S.A.

Gallery of Contemporary Noted Mycologists

57

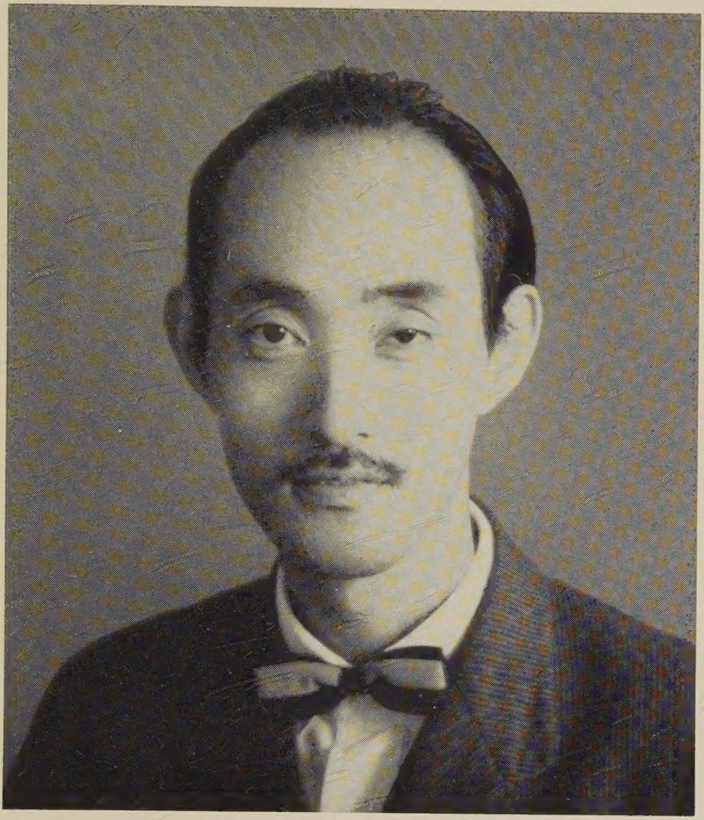


A. GONZÁLES OCHOA

Laboratorios de Micologia
Instituto de Salubridad y Enfermedades Tropicales
Mexico, D. F., Mexico

Gallery of Contemporary Noted Mycologists

59



Y. YASUDA

Department of Microbiology
Wakayama Prefectural Medical College
Wakayama-shi, Japan

Gallery of Contemporary Noted Mycologists

60